

Stem Cell and Life Immortal Research Literatures

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Abstract: The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the stem cell and life immortal related studies.

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Introduction

The stem cell is the origin of an organism's life that has the potential to develop into many different types of cells in life bodies. In many tissues stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. When a stem cell divides, each new cell has the potential either to remain a stem cell or become another type of cell with a more specialized function, such as a red blood cell or a brain cell. This article introduces recent research reports as references in the related studies.

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Boehm, A. M., K. Khalturin, et al. "FoxO is a critical regulator of stem cell maintenance in immortal Hydra." *Proc Natl Acad Sci U S A*. 2012 Nov 27;109(48):19697-702. doi: [10.1073/pnas.1209714109](https://doi.org/10.1073/pnas.1209714109). Epub 2012 Nov 12.

Hydra's unlimited life span has long attracted attention from natural scientists. The reason for that phenomenon is the indefinite self-renewal capacity of its stem cells. The underlying molecular mechanisms have yet to be explored. Here, by comparing the transcriptomes of Hydra's stem cells followed by functional analysis using transgenic polyps, we identified the transcription factor forkhead box O (FoxO) as one of the critical drivers of this continuous self-renewal. foxO overexpression increased interstitial stem cell and progenitor cell proliferation and activated stem cell genes in terminally differentiated somatic cells. foxO down-regulation led to an increase in the number of terminally differentiated cells, resulting in a drastically reduced population growth rate. In addition, it caused down-

regulation of stem cell genes and antimicrobial peptide (AMP) expression. These findings contribute to a molecular understanding of Hydra's immortality, indicate an evolutionarily conserved role of FoxO in controlling longevity from Hydra to humans, and have implications for understanding cellular aging.

Booth, B. W., C. A. Boulanger, et al. "Amphiregulin mediates self-renewal in an immortal mammary epithelial cell line with stem cell characteristics." *Exp Cell Res*. 2010 Feb 1;316(3):422-32. doi: [10.1016/j.yexcr.2009.11.006](https://doi.org/10.1016/j.yexcr.2009.11.006). Epub 2009 Nov 12.

Amphiregulin (AREG), a ligand for epidermal growth factor receptor, is required for mammary gland ductal morphogenesis and mediates estrogen actions in vivo, emerging as an essential growth factor during mammary gland growth and differentiation. The COMMA-D beta-geo (CDBetageo) mouse mammary cell line displays characteristics of normal mammary progenitor cells including the ability to regenerate a mammary gland when transplanted into the cleared fat pad of a juvenile mouse, nuclear label retention, and the capacity to form anchorage-independent mammospheres. We demonstrate that AREG is essential for formation of floating mammospheres by CDBetageo cells and that the mitogen activated protein kinase signaling pathway is involved in AREG-mediated mammosphere formation. Addition of exogenous AREG promotes mammosphere formation in cells where AREG expression is knocked down by siRNA and mammosphere formation by AREG(-/-) mammary epithelial cells. AREG knockdown inhibits mammosphere formation by duct-limited mammary progenitor cells but not lobule-limited mammary progenitor cells. These data demonstrate AREG mediates the function of a subset of mammary progenitor cells in vitro.

Cacci, E., A. Villa, et al. "Generation of human cortical neurons from a new immortal fetal neural stem cell line." Exp Cell Res. 2007 Feb 1;313(3):588-601. Epub 2006 Nov 7.

Isolation and expansion of neural stem cells (NSCs) of human origin are crucial for successful development of cell therapy approaches in neurodegenerative diseases. Different epigenetic and genetic immortalization strategies have been established for long-term maintenance and expansion of these cells in vitro. Here we report the generation of a new, clonal NSC (hc-NSC) line, derived from human fetal cortical tissue, based on v-myc immortalization. Using immunocytochemistry, we show that these cells retain the characteristics of NSCs after more than 50 passages. Under proliferation conditions, when supplemented with epidermal and basic fibroblast growth factors, the hc-NSCs expressed neural stem/progenitor cell markers like nestin, vimentin and Sox2. When growth factors were withdrawn, proliferation and expression of v-myc and telomerase were dramatically reduced, and the hc-NSCs differentiated into glia and neurons (mostly glutamatergic and GABAergic, as well as tyrosine hydroxylase-positive, presumably dopaminergic neurons). RT-PCR analysis showed that the hc-NSCs retained expression of Pax6, Emx2 and Neurogenin2, which are genes associated with regionalization and cell commitment in cortical precursors during brain development. Our data indicate that this hc-NSC line could be useful for exploring the potential of human NSCs to replace dead or damaged cortical cells in animal models of acute and chronic neurodegenerative diseases. Taking advantage of its clonality and homogeneity, this cell line will also be a valuable experimental tool to study the regulatory role of intrinsic and extrinsic factors in human NSC biology.

De Filippis, L., G. Lamorte, et al. "A novel, immortal, and multipotent human neural stem cell line generating functional neurons and oligodendrocytes." Stem Cells. 2007 Sep;25(9):2312-21. Epub 2007 Jun 7.

The discovery and study of neural stem cells have revolutionized our understanding of the neurogenetic process, and their inherent ability to adopt expansive growth behavior in vitro is of paramount importance for the development of novel therapeutics based on neural cell replacement. Recent advances in high-throughput assays for drug development and gene discovery dictate the need for rapid, reproducible, long-term expansion of human neural stem cells (hNSCs). In this view, the complement of wild-type cell lines currently available is insufficient. Here we report the establishment of a stable human neural stem cell line (immortalized

human NSCs [IhNSCs]) by v-myc-mediated immortalization of previously derived wild-type hNSCs. These cells demonstrate three- to fourfold faster proliferation than wild-type cells in response to growth factors but retain rather similar properties, including multipotentiality. By molecular biology, biochemistry, immunocytochemistry, fluorescence microscopy, and electrophysiology, we show that upon growth factor removal, IhNSCs completely downregulate v-myc expression, cease proliferation, and differentiate terminally into three major neural lineages: astrocytes, oligodendrocytes, and neurons. The latter are functional, mature cells displaying clear-cut morphological and physiological features of terminally differentiated neurons, encompassing mostly the GABAergic, glutamatergic, and cholinergic phenotypes. Finally, IhNSCs produce bona fide oligodendrocytes in fractions up to 20% of total cell number. This is in contrast to the negligible propensity of hNSCs to generate oligodendroglia reported so far. Thus, we describe an immortalized hNSC line endowed with the properties of normal hNSCs and suitable for developing the novel, reliable assays and reproducible high-throughput gene and drug screening that are essential in both diagnostics and cell therapy studies.

Grigoryan, G. A., J. A. Gray, et al. "Conditionally immortal neuroepithelial stem cell grafts restore spatial learning in rats with lesions at the source of cholinergic forebrain projections." Restor Neurol Neurosci. 2000;17(4):1.

Purpose: Loss of cholinergic projections from the basal forebrain (BF) to the cortex and from the medial septal area (MSA) to the hippocampus is a reliable correlate of cognitive deficits in aging and Alzheimer's disease (AD). We assessed the capacity of grafts of the conditionally immortal MHP36 clonal stem cell line to improve spatial learning in rats showing profound deficits after lesions to these projections. Methods: Rats were lesioned by infusions of S-AMPA unilaterally into BF or bilaterally into both BF and MSA. MHP36 cells were implanted ipsilaterally in cortex or basal forebrain two weeks after unilateral BF lesions, and in cortex and hippocampus bilaterally six months after bilateral BF-MSA lesions. Intact and lesion-only controls received vehicle. Six weeks later rats were assessed in spatial learning and memory tasks in the water maze, and then perfused for identification of grafted cells by beta-galactosidase immunohistochemistry. Results: Lesioned rats with MHP36 grafts, whether implanted two weeks or six months after lesioning, learned to

find a submerged platform in the water maze as rapidly as intact controls, and showed a strong preference for the platform quadrant on probe trials, whereas lesioned controls were impaired in all measures. Grafted cells of both neuronal and glial morphologies, migrated away from cortical implantation sites in BF Lesioned rats to the striatum, thalamus and basal forebrain lesion area. Cells implanted in basal forebrain showed a similar distribution. In rats with bilateral BF-MSA lesions, grafts implanted in the hippocampus migrated widely through all layers but cortical grafts largely escaped up the needle tract into the meninges. Conclusions: Although MHP36 grafts were functionally effective in both lesion models, the site and age of lesions and site of implantation influenced the pattern of engraftment. This flexibility encourages the development of conditionally immortal human stem cell lines with similar capacities for functional repair of variable neuronal degeneration in AD or aging.

Hodges, H., P. Sowinski, et al. "Functional reconstruction of the hippocampus: fetal versus conditionally immortal neuroepithelial stem cell grafts." Novartis Found Symp. 2000;231:53-65; discussion 65-9.

Late fetal CA1 hippocampal grafts and stem cell grafts from the conditionally immortal MHP36 clonal line derived from the H-2Kb-tsA58 transgenic mouse neuroepithelium both improved spatial deficits in rats with ischaemic CA1 damage induced by four-vessel occlusion (4VO). However, the distribution of fetal and MHP36 grafts differed. Fetal cells lodged in clumps around the implant sites and along the corpus callosum, whilst MHP36 grafts infiltrated the area of CA1 ischaemic damage, achieving apparent architectural reconstruction of the hippocampus. The migration of MHP36 cells is damage-dependent. Few cells were found in intact brain; after 15 min of 4VO cells repopulated only the discrete area of CA1 cell loss, whereas with more extensive damage after 30 min occlusion cells migrated to all hippocampal fields and to cortex. A higher proportion of grafted MHP36 cells differentiated into neurons in the host CA1 field than grafts of striatal or cortical expanded cell populations. Cortical population grafts were as effective as MHP36 grafts in improving water maze learning, whereas striatal or ventral mesencephalic cells were ineffective, indicating a degree of stem cell specificity. The efficacy of MHP36 cells extends to primates. In marmosets with profound impairments in conditional discrimination tasks after lesions of the CA1 field, MHP36 cells improved performance as effectively as fetal grafts and migrated evenly through the CA1 field, in contrast to clustered fetal cells. These findings suggest that MHP36 stem cell grafts are as

effective as fetal grafts in functional repair of hippocampal damage, and that their preference for areas of cell loss and adoption of appropriate morphologies is consistent with a point-to-point repair mechanism.

Hodges, H., T. Veizovic, et al. "Conditionally immortal neuroepithelial stem cell grafts reverse age-associated memory impairments in rats." Neuroscience. 2000;101(4):945-55.

In order to investigate the effects of stem cell grafts on water maze deficits in aged (22-month-old) rats, three groups of aged rats, assigned by pre-training latency scores to unimpaired, impaired control and impaired grafted groups, were compared with young (five-month-old) controls, six to eight weeks after implantation of cells from the conditionally immortal Maudsley hippocampal stem cell line, clone 36 (MHP36 stem cell line), in the cortex, striatum and hippocampus. Grafted rats were substantially superior to their matched impaired aged controls, and learned to find the platform as rapidly as unimpaired aged rats, although young controls were more efficient than all aged groups in several measures of spatial search during training. On the probe trial, however, aged rats with grafts showed significantly better recall of the precise position of the platform than any other group, including young controls, possibly indicating some perseveration. A further comparison found that groups of unimpaired and moderately impaired aged rats showed far less improvement from water maze pre-training to acquisition phases than young controls, indicative of progressive deficits over time. Histological investigation showed that beta-galactosidase-positive MHP36 cells migrated widely from the implantation sites to infiltrate the striatal matrix, all hippocampal fields and areas of the cortex. Grafted cells showed both astrocytic and neuronal morphologies, with cells of pyramidal and granular appearance in appropriate hippocampal strata. Taken together, these results indicate that neuroepithelial stem cell grafts extensively colonize the aged rat brain and substantially reverse progressive cognitive decline associated with ageing.

Kawa, Y., M. Ito, et al. Stem cell factor and/or endothelin-3 dependent immortal melanoblast and melanocyte populations derived from mouse neural crest cells, Pigment Cell Res. 2000;13 Suppl 8:73-80.

Stem cell factor (SCF) and endothelin-3 (ET3) are both necessary for melanocyte development. In order to obtain immortal cell populations of melanoblasts that can survive without feeder cells, we first obtained an immortal cell population of neural crest cells (NCCs) from SI/+ and +/+ mice of strain WB by incubating with a culture medium

supplemented with SCF and ET3, and then we designated them as NCC-SE3 cells. NCC-SE3 cells were bipolar, polygonal, or round in shape and possessed melanosomes of stages I-III (mainly stage I). They were positive to dihydroxyphenylalanine (DOPA) reaction and expressed KIT (a receptor tyrosine kinase), tyrosinase, tyrosinase-related protein-1 (TRP1), tyrosinase-related protein-2 (TRP2), and endothelin-B receptor (ETRB) as determined by immunostaining. We next cultured NCC-SE3 cells by changing culture medium from the one supplemented with SCF + ET3 to the one supplemented with SCF or ET3. NCC-SE3 cells cultured with ET3 alone, designated as NCC-E3 cells, were bipolar in shape and had mainly stage II melanosomes and expressed the same proteins as did NCC-SE3 cells. However, NCC-SE3 cells cultured with SCF alone, designated as NCC-S4.1 cells, were polygonal in shape and had mainly stage I melanosomes. They are thought to be more immature because they were positive to KIT, TRP1, and TRP2, but not to ETR(B), tyrosinase, and DOPA reaction. When 12-O-tetradecanoylphorbol 13-acetate and cholera toxin were added to the culture medium, NCC-S4.1 cells changed shape from polygonal to bipolar and became DOPA-positive. This suggests that NCC-S4.1 cells are melanoblasts that have the potential to differentiate into melanocytes. These cell populations will be extremely useful to study factors that affect melanocyte development and melanogenesis.

Kim, S. U., A. Nagai, et al. "Production and characterization of immortal human neural stem cell line with multipotent differentiation property." *Methods Mol Biol.* 2008;438:103-21. doi: [10.1007/978-1-59745-133-8_10](https://doi.org/10.1007/978-1-59745-133-8_10).

We document the protocols and methods for the production of immortalized cell lines of human neural stem cells from the human fetal central nervous system (CNS) cells by using a retroviral vector encoding v-myc oncogene. One of the human neural stem cell lines (HB1.F3) was found to express nestin and other specific markers for human neural stem cells, giving rise to three fundamental cell types of the CNS: neurons, astrocytes, and oligodendrocytes. After transplantation into the brain of mouse model of stroke, implanted human neural stem cells were observed to migrate extensively from the site of implantation into other anatomical sites and to differentiate into neurons and glial cells.

Koziol, U., T. Rauschendorfer, et al. "The unique stem cell system of the immortal larva of the human parasite *Echinococcus multilocularis*." *Evodevo.* 2014 Mar 6;5(1):10. doi: [10.1186/2041-9139-5-10](https://doi.org/10.1186/2041-9139-5-10).

BACKGROUND: It is believed that in tapeworms a separate population of undifferentiated cells, the germinative cells, is the only source of cell proliferation throughout the life cycle (similar to the neoblasts of free living flatworms). In *Echinococcus multilocularis*, the metacestode larval stage has a unique development, growing continuously like a mass of vesicles that infiltrate the tissues of the intermediate host, generating multiple protoscoleces by asexual budding. This unique proliferation potential indicates the existence of stem cells that are totipotent and have the ability for extensive self-renewal. **RESULTS:** We show that only the germinative cells proliferate in the larval vesicles and in primary cell cultures that undergo complete vesicle regeneration, by using a combination of morphological criteria and by developing molecular markers of differentiated cell types. The germinative cells are homogeneous in morphology but heterogeneous at the molecular level, since only sub-populations express homologs of the post-transcriptional regulators nanos and argonaute. Important differences are observed between the expression patterns of selected neoblast marker genes of other flatworms and the *E. multilocularis* germinative cells, including widespread expression in *E. multilocularis* of some genes that are neoblast-specific in planarians. Hydroxyurea treatment results in the depletion of germinative cells in larval vesicles, and after recovery following hydroxyurea treatment, surviving proliferating cells grow as patches that suggest extensive self-renewal potential for individual germinative cells. **CONCLUSIONS:** In *E. multilocularis* metacestodes, the germinative cells are the only proliferating cells, presumably driving the continuous growth of the larval vesicles. However, the existence of sub-populations of the germinative cells is strongly supported by our data. Although the germinative cells are very similar to the neoblasts of other flatworms in function and in undifferentiated morphology, their unique gene expression pattern and the evolutionary loss of conserved stem cell regulators suggest that important differences in their physiology exist, which could be related to the unique biology of *E. multilocularis* larvae.

Merok, J. R., J. A. Lansita, et al. "Cosegregation of chromosomes containing immortal DNA strands in cells that cycle with asymmetric stem cell kinetics." *Cancer Res.* 2002 Dec 1;62(23):6791-5.

A long-standing intriguing hypothesis in cancer biology is that adult stem cells avoid mutations from DNA replication errors by a unique pattern of chromosome segregation. At each asymmetric cell division, adult stem cells have been postulated to selectively retain a set of chromosomes that contain old template DNA strands (i.e., "immortal DNA

strands"). Using cultured cells that cycle with asymmetric cell kinetics, we confirmed both the existence of immortal DNA strands and the cosegregation of chromosomes that bear them. Our findings also lead us to propose a role for immortal DNA strands in tissue aging as well as cancer.

Pollock, K., P. Stroemer, et al. "A conditionally immortal clonal stem cell line from human cortical neuroepithelium for the treatment of ischemic stroke." Exp Neurol. 2006 May;199(1):143-55. Epub 2006 Feb 7.

Transplantation of neural stem cells into the brain is a novel approach to the treatment of chronic stroke disability. For clinical application, safety and efficacy of defined, stable cell lines produced under GMP conditions are required. To this end, a human neural stem cell line, CTX0E03, was derived from human somatic stem cells following genetic modification with a conditional immortalizing gene, c-mycER(TAM). This transgene generates a fusion protein that stimulates cell proliferation in the presence of a synthetic drug 4-hydroxy-tamoxifen (4-OHT). The cell line is clonal, expands rapidly in culture (doubling time 50-60 h) and has a normal human karyotype (46 XY). In the absence of growth factors and 4-OHT, the cells undergo growth arrest and differentiate into neurons and astrocytes. Transplantation of CTX0E03 in a rat model of stroke (MCAo) caused statistically significant improvements in both sensorimotor function and gross motor asymmetry at 6-12 weeks post-grafting. In addition, cell migration and long-term survival in vivo were not associated with significant cell proliferation. These data indicate that CTX0E03 has the appropriate biological and manufacturing characteristics necessary for development as a therapeutic cell line.

Snoeck, H. W. "Can Metabolic Mechanisms of Stem Cell Maintenance Explain Aging and the Immortal Germline?" Cell Stem Cell. 2015 Jun 4;16(6):582-4. doi: 10.1016/j.stem.2015.04.021.

The mechanisms underlying the aging process are not understood. Even tissues endowed with somatic stem cells age while the germline appears immortal. I propose that this paradox may be explained by the pervasive use of glycolysis by somatic stem cells as opposed to the predominance of mitochondrial respiration in gametes.

Walters, K. "Colonic stem cell data are consistent with the immortal model of stem cell division under non-random strand segregation." Cell Prolif. 2009 Jun;42(3):339-47. doi: 10.1111/j.1365-2184.2009.00600.x. Epub 2009 Mar 27.

OBJECTIVES: Colonic stem cells are thought to reside towards the base of crypts of the colon, but their numbers and proliferation mechanisms are not well characterized. A defining property of stem cells is that they are able to divide asymmetrically, but it is not known whether they always divide asymmetrically (immortal model) or whether there are occasional symmetrical divisions (stochastic model). By measuring diversity of methylation patterns in colon crypt samples, a recent study found evidence in favour of the stochastic model, assuming random segregation of stem cell DNA strands during cell division. Here, the effect of preferential segregation of the template strand is considered to be consistent with the 'immortal strand hypothesis', and explore the effect on conclusions of previously published results. **MATERIALS AND METHODS:** For a sample of crypts, it is shown how, under the immortal model, to calculate mean and variance of the number of unique methylation patterns allowing for non-random strand segregation and compare them with those observed. **RESULTS:** The calculated mean and variance are consistent with an immortal model that incorporates non-random strand segregation for a range of stem cell numbers and levels of preferential strand segregation. **CONCLUSIONS:** Allowing for preferential strand segregation considerably alters previously published conclusions relating to stem cell numbers and turnover mechanisms. Evidence in favour of the stochastic model may not be as strong as previously thought.

Walters, K. "Parameter estimation for an immortal model of colonic stem cell division using approximate Bayesian computation." J Theor Biol. 2012 Aug 7;306:104-14. doi: 10.1016/j.jtbi.2012.04.021. Epub 2012 Apr 26.

In this paper we use approximate Bayesian computation to estimate the parameters in an immortal model of colonic stem cell division. We base the inferences on the observed DNA methylation patterns of cells sampled from the human colon. Utilising DNA methylation patterns as a form of molecular clock is an emerging area of research and has been used in several studies investigating colonic stem cell turnover. There is much debate concerning the two competing models of stem cell turnover: the symmetric (immortal) and asymmetric models. Early simulation studies concluded that the observed methylation data were not consistent with the immortal model. A later modified version of the immortal model that included preferential strand segregation was subsequently shown to be consistent with the same methylation data. Most of this earlier work assumes site independent methylation models that do not take account of the known processivity of methyltransferases whilst other work does not take into account the methylation errors

that occur in differentiated cells. This paper addresses both of these issues for the immortal model and demonstrates that approximate Bayesian computation provides accurate estimates of the parameters in this neighbour-dependent model of methylation error rates. The results indicate that if colonic stem cells divide asymmetrically then colon stem cell niches are maintained by more than 8 stem cells. Results also indicate the possibility of preferential strand segregation and provide clear evidence against a site-independent model for methylation errors. In addition, algebraic expressions for some of the summary statistics used in the approximate Bayesian computation (that allow for the additional variation arising from cell division in differentiated cells) are derived and their utility discussed.

The above contents are the collected information from Internet and public resources to offer to the people for the convenient reading and information disseminating and sharing.

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