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Journal of Cell Biology (*JCB*) and *Journal of Experimental Medicine* (*JEM*) editors are delighted to present this selection of recent, cutting-edge stem cell research. *JCB* publishes new cellular and molecular advances in any area of basic cell biology as well as papers that describe applied cell biology to translational fields such as stem cells. *JEM* is interested in original findings on all aspects of disease pathogenesis, including both hematopoietic stem cell and non-hematopoietic stem cell physiopathology. This collection includes some of our most highly read papers and articles selected to reflect the breadth of stem cell research published in our journals. We hope you enjoy these articles that delve into the cell biology of regeneration, stem cell division, fate decision, and their links to various diseases and innovative therapies.

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Brochure articles: Ben Short, PhD and Christina Szalinski, PhD

Design: Christine Candia

On the cover: Two-photon images of $Hras^{G12V/+}$ hair follicles during the "second rest phase" (P55). Mosaic activation of Hras using lentiviral-Cre delivery demonstrates that wild-type follicles (green) within the $Hras^{G12V/+}$ skin reenter the growth phase precociously along with their Hras mutant (red) neighbors. Image © 2019 Pineda et al. Read "Hair follicles restrict cancer growth," page 8.

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MYC REGULATES RETINAL REGENERATION

Study identifies multiple roles for Myc in controlling the induction and proliferation of Muller glia-derived progenitor cells upon retinal injury in zebrafish

Compared with humans and other mammals, zebrafish have a much greater capacity to regenerate damaged tissues after injury. Damage to the zebrafish retina, for example, causes Muller glial cells to dedifferentiate into multipotent progenitor cells that proliferate and eventually redifferentiate into many different retinal cell types, thereby enabling the tissue's regeneration.

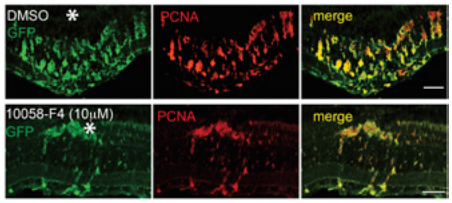
Muller glia with some stem cell–like characteristics have been identified in mammalian retina, but their regenerative capacity appears to be limited. "Unraveling the complete cascade of gene regulatory networks induced by retina injury in zebrafish could help in deciphering the lack of efficient regeneration in mammals," explains Rajesh Ramachandran from the Indian Institute of Science Education and Research in Mohali.

Ramachandran has previously identified important roles for several factors in Muller glia dedifferentiation and retinal regeneration, such as the transcriptional activator Ascl1a and one of its targets, *lin28a*. In their latest study, Ramachandran and colleagues, including first author Soumitra Mitra, investigated a potential role for the transcription factor Myc, which, among many biological functions, can promote the dedifferentiation of many cell types to form induced pluripotent stem cells.

Mitra et al. found that, upon retinal injury, the two zebrafish Myc genes, *myca* and *mycb*, were rapidly induced in both Muller glia-derived progenitor cells (MGPCs) and neighboring cells at the injury site. Knocking down or inhibiting Myc impaired the dedifferentiation of Muller glia into MGPCs and blocked MGPC proliferation and retinal regeneration.

The researchers found that *mycb* promotes the generation and proliferation of MGPCs by inducing the expression of *ascl1a,* leading, in turn, to the production of *lin28a.* (This gene regulatory cascade is tightly controlled: while Ascl1a feeds back to promote further *mycb* expression, another transcriptional target of Ascl1a, Insm1a, represses *mycb*).

In neighboring cells, however, Mycb recruits the histone deacetylase Hdac1 to repress the *lin28a* promoter and limit *lin28a* expression. This may help prevent MGPCs from forming throughout the retina and restrict their appearance to the immediate site of injury. The researchers found that Notch signaling plays a key role in this restriction by inducing the expression of an effector called *her4.1* (itself a target for Mycb and Hdac1).



Four days after injury, the number of proliferative MGPCs (labeled with GFP, green, and PCNA, red) is reduced in a zebrafish retina treated with 10058-F4, a drug that inhibits the interaction between Myc and its obligatory transcriptional partner Max. Credit: Mitra et al., 2019

Though the precise details remain to be determined, Mycb therefore functions as both a transcriptional activator and repressor to finetune the extent of *lin28a* expression and MGPC formation in response to retinal injury.

"Our study suggests that Mycs are part of a gene regulatory network essential for retina regeneration, and provides insights that could ultimately help improve Muller glia reprogramming in injured human retina and enable successful repair," Ramachandran says.

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Mitra, S., P. Sharma, S. Kaur, M.A. Khursheed, S. Gupta, M. Chaudhary, A.J. Kurup, and R. Ramachandran. 2019. Dual regulation of *lin28a* by Myc is necessary during zebrafish retina regeneration. *J. Cell Biol.* 218:489–507.
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THE RNA EXOSOME RESTRAINS HESC DIFFERENTIATION

The nuclease complex degrades a variety of RNAs to maintain pluripotency and suppress the differentiation of human embryonic stem cells

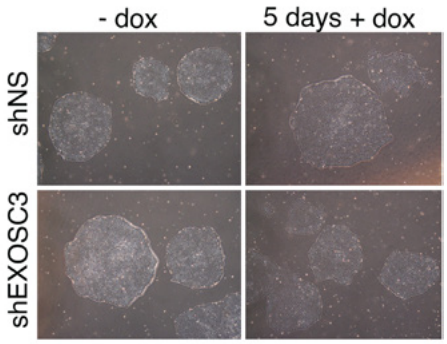
Human embryonic stem cells (hESCs) can differentiate into all three embryonic germ layers: the endoderm, mesoderm, and ectoderm. This pluripotent state is maintained by a network of transcriptional regulators and epigenetic factors that allow the cells to self-renew indefinitely while remaining poised to differentiate rapidly when required. For example, the chromatin in hESCs is in a less condensed "open" state amenable to gene expression.

"hESCs must balance the need to keep many genes transcriptionally competent with the need to protect themselves from deleterious consequences of promiscuous transcription," explains Sandra Wolin from the National Cancer Institute. Various RNA surveillance and degradation pathways might limit the damage of any prematurely expressed RNAs in hESCs. The RNA exosome, for example, is a multiprotein nuclease complex involved in RNA quality control that has also been proposed to regulate the levels of specific mRNAs involved in cell proliferation and development. But whether it helps maintain hESC pluripotency was unknown.

Wolin and colleagues, including first author Cedric Belair, depleted the RNA exosome from hESCs by targeting one of the complex's core subunits, EXOSC3. hESCs lacking EXOSC3 still expressed the pluripotency markers OCT4, NANOG, and SOX2. When induced to differentiate, however, EXOSC3-deficient hESCs expressed increased levels of endodermal, mesodermal, and ectodermal markers, suggesting that loss of the RNA exosome expedites hESC differentiation into all three germ layers. Accordingly, the researchers found, wild-type hESCs downregulate several subunits of the nuclease complex during differentiation. Conversely, differentiated cells upregulate these subunits when they are reprogrammed into induced pluripotent stem cells.

Wolin and colleagues identified RNAs of various classes that bind to the exosome in hESCs and are upregulated when the nuclease complex is depleted. These direct targets of the exosome include LINE-1 retrotransposons and a long noncoding RNA that regulates the transcription start site of a transcription factor important for hESC pluripotency. The RNA exosome also restricts the levels of a primary microRNA, pri-miR-205, that is linked to endoderm development and numerous mRNAs encoding developmental regulators such as the transcription factor FOXH1.

hESCs require FOXH1 to induce formation of the mesendoderm, the precursor to the mesodermal and endodermal germ layers. Wolin and colleagues found that, in the absence of the RNA exosome, the transcriptional targets of FOXH1 are elevated during hESC differentiation, but these increases were abolished by FOXH1 depletion, indicating that the exosome restrains mesendoderm formation by degrading *FOXH1* mRNA.



Depletion of the RNA exosome has little effect on the appearance (or pluripotency) of hESC colonies. Upon differentiation, however, endoderm, mesoderm, and ectoderm markers are all increased in the absence of the exosome, indicating that the nuclease complex restrains hESC differentiation into all three germ layers.
Credit: Belair et al., 2019

"Our data support a model in which the exosome contributes to hESC pluripotency by degrading RNAs that encode key developmental regulators," Wolin says. "Moreover, because the exosome can undergo rapid inactivation, it may function as a developmental switch that allows hESCs to quickly increase levels of important RNAs."

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Belair, C., S. Sim, K.-Y. Kim, Y. Tanaka, I.-H. Park, and S.L. Wolin. 2019. The RNA exosome nuclease complex regulates human embryonic stem cell differentiation. *J. Cell Biol.* 218:2564–2582.
https://doi.org/10.1083/jcb.201811148

HAIR FOLLICLES RESTRICT CANCER GROWTH

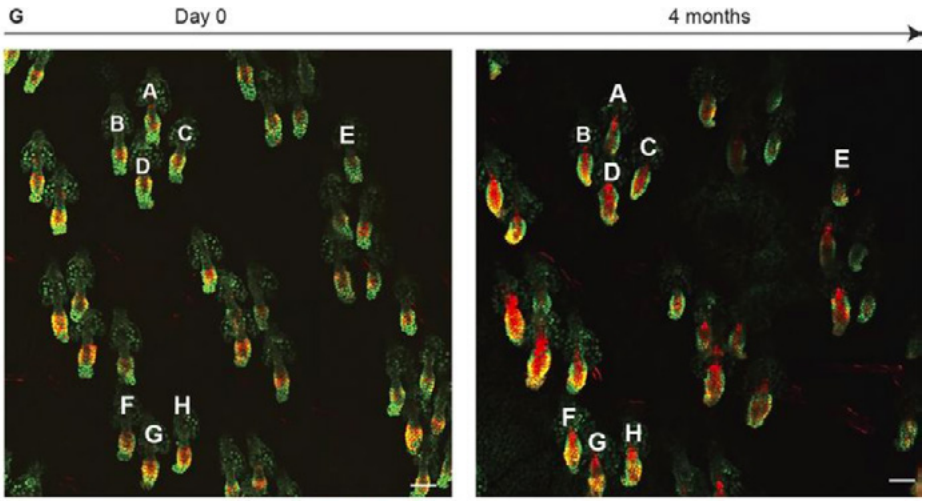
Normal skin corrals cancer-causing mutations and reveals how tissue can subvert tumorigenesis

Normal aged skin contains cancer-causing mutations, recent research has shown, but how these mutations are prevented from forming tumors was a mystery. Christiana M. Pineda, Valentina Greco, and colleagues at Yale University used unique live-tissue imaging to track mouse skin cells after inducing cancer-causing mutations. They found that protection against skin cancer comes from a surprising place: hair follicles.

About 30% of all cancers contain a Ras mutation, but these same mutations have been found in non-cancerous skin epithelia. Pineda and colleagues induced Ras mutations in mouse hair follicle cells along with a glowing red reporter to track the mutant stem cells and their progeny. The cells persisted in the epithelium, revealing that the body doesn't just eliminate mutant cells.

Pineda and colleagues found that when they induced mutations in hair follicle stem cells, they outcompeted wild-type neighboring cells. They still responded to normal tissue constraints, such as resting phase cues, however. Even after a year, the transformed cells did not develop into tumors. In contrast, targeting the Ras mutation to the upper non-cycling region of the skin epithelium led to benign outgrowths.

Introducing a second mutation that results in the loss of TGFβ signaling into Ras-mutant hair follicle stem cells did induce some tumors, typically at sites of high grooming or scratching. Imaging revealed that those tumors arose after an injury caused them to exit the follicular niche. To test whether injury can promote tumorigenesis within the follicle, they ablated hair follicle bulbs and the double mutant cells showed rapid, and normal, regeneration. "Once out of the follicular niche, Hras mutant cells can no longer be controlled and contained through hair regeneration programs," Pineda says.



Two-photon images of the ear skin from the same mouse at day 0 and 4 months after Ras activation reveal normal follicular architecture despite the persistent presence of mutant cells (red).
Credit: Pineda et al., 2019

"Our results indicate that the hair follicle has a unique ability to cope with Ras-activated cells. This organ is able to integrate the mutant epithelial cells while remaining clinically normal," Pineda says. There's still much to learn about what's going on in the skin that could be applied to other cancers. "Manipulation of certain cell types or signaling pathways may enable and/or enhance the ability of other epithelial tissues to also suppress oncogenic growth."

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https://doi.org/10.1083/jcb.201907178

CENTROMERES DRIVE ASYMMETRIC STEM CELL DIVISION

Asymmetric incorporation of the centromeric histone CENP-A during G2/M regulates the fate of *Drosophila* female germline stem cells

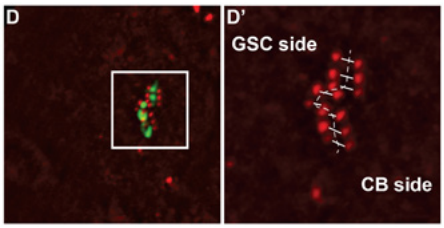
Stem cells divide asymmetrically to produce one new daughter stem cell and one daughter cell that will subsequently differentiate. One mechanism that has been proposed to contribute to this asymmetry is the selective inheritance of sister chromatids that carry specific epigenetic marks between the stem and daughter cell. Upon division, distinct epigenetic marks on sister chromatids can result in differential gene expression in the two resulting daughter cells. According to this "silent sister hypothesis," the centromeric regions of each sister chromatid—which are crucial for cell division and are epigenetically defined by the histone H3 variant CENP-A—would also be distinct to facilitate their selective segregation.

In human cells, CENP-A is assembled into centromeres at the end of mitosis. "However, centromere assembly dynamics can differ among metazoans and also among different cell types in the same organism," says Elaine Dunleavy from the National University of Ireland Galway. "To date, little is known about centromere assembly dynamics and functions during stem cell asymmetric divisions."

Dunleavy's team, including first author Ada Dattoli, followed the dynamics of centromere assembly in *Drosophila* female germline stem cells (GSCs) and found that, in these cells, CENP-A is deposited at centromeres after DNA replication but before chromosome segregation (i.e., during the G2/M transition). The researchers saw similar dynamics in *Drosophila* neural stem cells as well.

Dattoli, Dunleavy, and colleagues found that the cell cycle regulator CYCLIN A promotes CENP-A deposition in GSCs, whereas CYCLIN B, and its downstream target HASPIN kinase, prevent excessive CENP-A incorporation. But CENP-A isn't deposited evenly on each set of sister chromatids: superresolution microscopy revealed that more CENP-A is incorporated into the centromeres of chromatids that are destined to be segregated into the daughter cell that maintains its stem cell identity. Accordingly, these chromatids assembled larger kinetochores on their centromeres and captured more spindle microtubules, providing a potential mechanism to bias chromosome segregation.

Overexpressing CENP-A in GSCs, or depleting HASPIN kinase, caused CENP-A to be evenly deposited on sister chromatids. This is likely to result in the random segregation of sister chromatids, which, according to the silent sister hypothesis, would promote stem cell self-renewal. Indeed, overexpressing CENP-A or depleting HASPIN increased the overall number of GSCs in fly ovaries.



Superresolution image of a metaphase GSC (captured using the H3T3P marker, green) shows asymmetric levels of CENP-A (red) at the centromeres of sister chromatids. Centromeres of the chromatids destined for the daughter GSC have approximately 20% more CENP-A compared to centromeres of the chromatids destined to be inherited by the cystoblast (CB) that will undergo differentiation.
Credit: Dattoli et al., 2020

"Our results provide the first functional evidence that centromeres have a role in the epigenetic pathway that specifies stem cell identity," Dunleavy says. "Furthermore, our data support the silent sister hypothesis in which centromeres drive the selective segregation of sister chromatids and the asymmetric division of stem cells."

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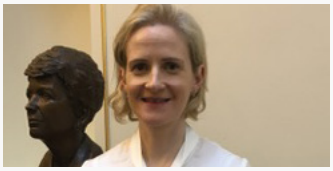

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Dattoli, A.A., B.L. Carty, A.M. Kochendoerfer, C. Morgan, A.E. Walshe, and E.M. Dunleavy. 2020. Asymmetric assembly of centromeres epigenetically regulates stem cell fate. *J. Cell Biol.* 219:e201910084.
https://doi.org/10.1083/jcb.201910084

NCAM REGULATES NEURAL PROGENITOR CELL FATE

Neural cell adhesion molecule controls the proliferation and differentiation of cortical progenitors by binding to the actin polymerization regulator profilin2

Development of the cerebral cortex depends on a precisely coordinated program of neural progenitor cell (NPC) proliferation, migration, and differentiation. The developmental program initially generates cortical neurons before switching to the production of astrocytes and, after birth, oligodendrocytes.

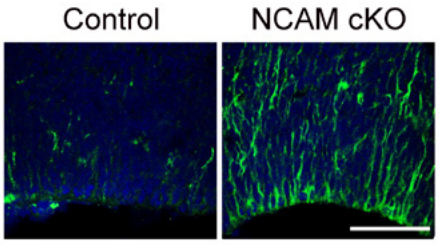
Neural cell adhesion molecule (NCAM) has a variety of functions within the nervous system and misexpression of this protein can alter the proliferation and differentiation of NPCs. "However, it was unknown whether NCAM is an intrinsic modulator of NPCs during cortical development," says Shen Li from Dalian Municipal Central Hospital in China.

Li and colleagues, including co-corresponding author Quan-Hong Ma from Soochow University, found that NCAM is prominently expressed in mouse NPCs during the early, neurogenic stages of cortical development, but its levels decline during the later, gliogenic periods when astrocytes and oligodendrocytes are produced. NPC-specific deletion of the NCAM gene caused a transient reduction in NPC proliferation and a delay in cortical neuron generation, while astrocyte and oligodendrocyte formation occurred earlier than normal.

To understand how NCAM might regulate NPC proliferation and differentiation, the researchers performed a yeast two-hybrid screen and found that an intracellular region of NCAM140, a splice variant of NCAM, binds to profilin2, a key regulator of actin polymerization. Profilin2 showed a similar dynamic expression pattern in NPCs during cortical development, and depleting the protein altered NPC proliferation and differentiation in vitro, favoring glial cell formation at the expense of neuron production.

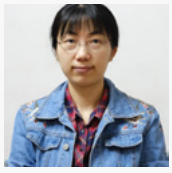
Li and colleagues confirmed that NCAM regulates the proliferation and fate of NPCs by binding to profilin2 and promoting actin polymerization. F-actin levels were reduced in NCAM-deficient NPCs and an NCAM mutant unable to bind to profilin2 was unable to rescue actin dynamics or normalize NPC proliferation and differentiation. NCAM-modulated actin dynamics may influence NPCs in several ways. One effect noticed by Li and colleagues is that mitotic NPCs fail to round up in NCAM-deficient mice, which is likely to impair the cells' progression through the cell cycle.

Single nucleotide polymorphisms in the *NCAM* gene and/or defects in NCAM proteolysis or glycosylation have been linked to a variety of neurological disorders, including autism. "Our study suggests that abnormalities in temporal NPC fate decision may contribute to the pathophysiology of neurodevelopmental diseases associated with abnormal NCAM function," Li says. "Understanding the molecular mechanisms underlying these abnormalities may help in the design of future strategies aimed at correcting neural differentiation in the affected brain."



At embryonic day 16, there are many more glial fibrillary acidic protein–positive ($GFAP^+$) astrocytes (green) in the developing cortex of a mouse whose NPCs lack NCAM (right).
Credit: Huang et al., 2020

RESEARCHER DETAILS



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Huang, R., D.-J. Yuan, S. Li, X.-S. Liang, Y. Gao, X.-Y. Lan, H.-M. Qin, Y.-F. Ma, G.-Y. Xu, M. Schachner, V. Sytnyk, J. Boltze, Q.-H. Ma, and S. Li. 2020. NCAM regulates temporal specification of neural progenitor cells via profilin2 during corticogenesis. *J. Cell Biol.* 219:e201902164.
https://doi.org/10.1083/jcb.201902164

GLUTAMYLATION REGULATES HSC SELF-RENEWAL

Reversible modification of the tumor suppressor BAP1 controls hematopoietic stem cell self-renewal and blood cell formation

The functions of proteins within cells can be altered by a wide range of reversible, posttranslational modifications, such as methylation, phosphorylation, and ubiquitination. Protein glutamylation—in which glutamate side chains are added to the γ-carboxyl groups of glutamic acid residues—was originally shown to regulate the dynamics of microtubules. More recently, however, the tubulin tyrosine ligase-like (TTLL) enzymes that add glutamate side chains, and the cytosolic carboxypeptidase (CCP) enzymes that remove them, have been shown to target a variety of proteins and regulate a range of cellular processes.

For example, Zusen Fan, Yong Tian, and colleagues at the Institute of Biophysics, Chinese Academy of Sciences, have previously shown that glutamylation regulates the development of both megakaryocytes and group 3 innate lymphoid cells. "However, how glutamylation regulates hematopoietic stem cells (HSCs), which give rise to all blood cell lineages, is unclear," says Fan.

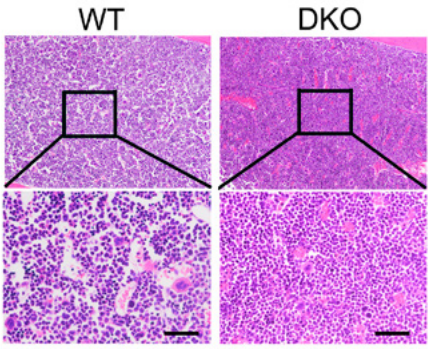
Fan and colleagues found that the deglutamylating enzyme CCP3 is highly expressed in mouse HSCs. Inhibiting this enzyme, or deleting the *Ccp3* gene, impaired HSC self-renewal and hematopoiesis, resulting in reduced HSC numbers in the bone marrow and decreased peripheral blood cell counts.

The researchers identified the nuclear deubiquitinase BAP1 as a key target of CCP3 in HSCs. The team found that glutamylation of BAP1 by the enzymes TTLL5 and TTLL7 accelerates the protein's degradation, whereas deglutamylation by CCP3 stabilizes BAP1.

BAP1 is a tumor suppressor linked to a range of cancers as well as the rare blood disorder myelodysplastic syndrome. Fan and colleagues found that BAP1 promotes HSC self-renewal and hematopoiesis by facilitating expression of the transcription factor *Hoxa1*. BAP1 and *Hoxa1* levels are reduced in *Ccp3*-deficient HSCs, limiting the stem cells' ability to remain quiescent and self-renew. The HSC pool is therefore soon exhausted, impairing hematopoiesis in *Ccp3*-knockout mice.

In contrast, HSCs from mice lacking TTLL5 and TTLL7 showed increased levels of BAP1 and Hoxa1, promoting HSC quiescence and self-renewal, as well as boosting hematopoiesis.

"Therefore, glutamylation and deglutamylation of BAP1 play a critical role in the regulation of HSC self-renewal and hematopoiesis," Fan says. "We are currently exploring the molecular mechanism by which BAP1 glutamylation modulates *Hoxa1* expression in a direct or indirect manner in HSCs."



Compared with wild-type (left), the bone marrow of mice lacking the glutamylating enzymes TTLL5 and TTLL7 (DKO, right) contains an increased number of cells due to an increase in BAP1 levels that promotes HSC self-renewal and hematopoiesis.
Credit: Xiong et al., 2020

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Xiong, Z., P. Xia, X. Zhu, J. Geng, S. Wang, B. Ye, X. Qin, Y. Qu, L. He, D. Fan, Y. Du, Y. Tian, and Z. Fan. 2020. Glutamylation of deubiquitinase BAP1 controls self-renewal of hematopoietic stem cells and hematopoiesis. *J. Exp. Med.* 217:e20190974.
https://doi.org/10.1084/jem.20190974

THE CHROMATIN LANDSCAPE OF GLIOBLASTOMA

Profiling of glioblastoma stem cells and primary tumor samples identifies subgroup-specific transcriptional regulatory circuits and novel therapeutic targets

Glioblastoma is the most common form of primary brain tumor and is largely incurable. Driven by therapy-resistant glioblastoma stem cells (GSCs), glioblastomas show high levels of phenotypic heterogeneity both between and within individual tumors.

Cellular states are generally defined by a small number of master transcription factors that bind to super-enhancers (SEs), driving the expression of numerous genes essential for establishing cellular identity, including the master transcription factors themselves. To define the core regulatory circuits that define glioblastoma cell states, a team of researchers led by Jeremy Rich and Xiuxing Wang at the University of California, San Diego, and Charles Lin, Stephen Mack, and Irtisha Singh at Baylor College of Medicine, profiled a large cohort of patient-derived GSCs and primary glioblastoma tumor samples.

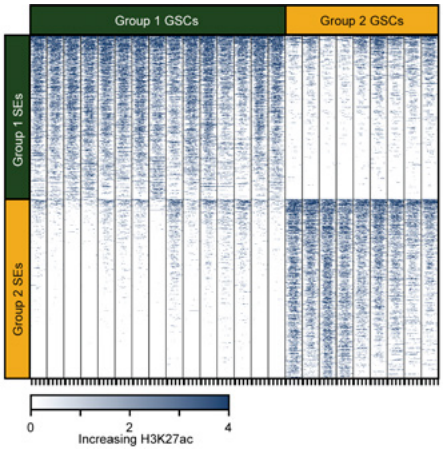
"We mapped active enhancer landscapes and integrated this with profiles of gene expression, DNA methylomes, copy number variations, and whole exomes to identify the core transcription factors and other SE-associated genes that establish and maintain GSC identity," Rich explains.

The researchers uncovered a set of SEs and genes active in >75% of GSCs, allowing them to derive a core gene signature for these cells. Tumors with high expression of this GSC signature showed increased malignancy and poor prognosis. Moreover, knocking down many of these SE-regulated genes impaired GSC proliferation in vitro.

In addition to this core signature common to the vast majority of GSCs, further analysis revealed that patient-derived GSCs cluster into at least two different groups with distinct chromatin signatures defined by unique SE activities and transcription factors. These different GSC states could also be identified in primary tumor samples from glioblastoma patients.

Knocking down group-specific transcription factors had group-specific effects. For example, knocking down RUNX2, a transcription factor uniquely active in group 2 GSCs, inhibited the growth of tumors derived from group 2 GSCs, but had no effect on tumors derived from group 1 GSCs.

"Transcription factors are notoriously challenging to target therapeutically," says Rich. "However, because these transcription factors are associated with SEs, we were able to identify other essential genes that are more amenable to therapeutic targeting." For example, the researchers found that group 1 GSCs show SE-mediated activation of MAPK/ERK signaling and can thus be specifically targeted by a small molecule inhibitor against this pathway.



Mapping histone H3 acetylation reveals the distinct patterns of SE activity in group 1 and group 2 GSCs. Credit: Mack et al., 2019

"In sum, our data reveal the existence of distinct GSC populations defined by unique chromatin signatures arising from specific regulatory circuits composed of SEs and SE-associated transcription factors," Rich says. "These SE programs that regulate GSC identity (both core and group specific) can be used for the discovery of new therapeutic targets."

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ORIGINAL PAPER

Mack, S.C., I. Singh, X. Wang, R. Hirsch, Q. Wu, R. Villagomez, J.A. Bernatchez, Z. Zhu, R.C. Gimple, L.J.Y. Kim, A. Morton, S. Lai, Z. Qiu, B.C. Prager, K.C. Bertrand, C. Mah, W. Zhou, C. Lee, G.H. Barnett, M.A. Vogelbaum, A.E. Sloan, L. Chavez, S. Bao, P.C. Scacheri, J.L. Siqueira-Neto, C.Y. Lin, and J.N. Rich. 2019. Chromatin landscapes reveal developmentally encoded transcriptional states that define human glioblastoma. *J. Exp. Med.* 216:1071–1090.
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PRIMITIVE HSCS CONTINUE CYCLING INTO ADULTHOOD

Study reveals that, contrary to a previous report, the most primitive hematopoietic stem cells do not become permanently quiescent and, instead, show continuous mitotic activity in adult mice

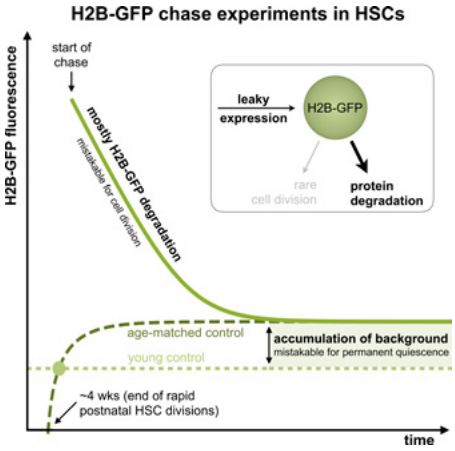
Hematopoietic stem cells (HSCs) are multipotent adult stem cells that can give rise to every type of blood cell throughout an individual's lifetime. The mitotic activity of HSCs can be tracked in vivo using an inducible "pulse" of fluorescent histone fusion protein (H2B-FP) which labels the cells' chromatin. This is followed by a "chase" period during which quiescent HSCs retain the fluorescent label while proliferating HSCs pass it on to their daughters, gradually diluting the fluorescent signal within a few cell divisions.

In 2016, a high-profile study used this approach in mice to identify a small population of primitive HSCs containing all of the HSC compartment's long-term repopulating activity. These cells appeared to undergo four self-renewing divisions during the first year of life before entering a state of permanent quiescence, apparently allowing them to retain fluorescent histone labeling for as long as 22 months. Based on this, the authors hypothesized that HSCs count and remember cell divisions. "However, their model considered neither the impact of continuous leaky background expression of the fluorescent histone label, nor the division-independent loss of fluorescence due to protein degradation," says Alexander Gerbaulet from the Institute for Immunology at TU Dresden.

Gerbaulet and colleagues, including first author Mina Morcos and co-corresponding author Thomas Zerjatke, found that in three different H2B-FP mouse strains—including the one used in the 2016 study—leaky expression even in the absence of induction causes fluorescent histone label to gradually accumulate in HSCs. This age-dependent background fluorescence accumulation was particularly prominent in quiescent HSCs with high repopulating activity. "We argue that this accumulated background can be easily misinterpreted as stable retention of induced label," Zerjatke says.

On the other hand, Gerbaulet and colleagues found that the half-life of fluorescent histones is within the range of 2–6 weeks, meaning that protein degradation represents the major contributor to loss of fluorescenct label in rarely dividing cells. "This precludes very long chase periods and neglecting label degradation will result in a massive overestimation of the divisional activity of quiescent HSCs," says Morcos.

The researchers built a new mathematical model for pulse-chase labeling of HSCs with fluorescent histones, incorporating the impact of leaky background expression and protein degradation. The model successfully recapitulated experimental observations, including the preferential accumulation of background fluorescence in more quiescent HSCs. Using their model to infer the mitotic activity of HSCs, Gerbaulet and colleagues saw no evidence for HSCs counting and remembering a set number of divisions before abruptly entering permanent quiescence. "Instead, our data suggest that primitive HSCs continue to cycle in aged mice, undergoing infrequent but steady mitotic divisions," Gerbaulet says.



Scheme recapitulates key features of pulse-chase experiments to identify quiescent HSCs retaining H2B-GFP label. Infrequently dividing HSCs dilute fluorescent label mostly by protein degradation. In parallel, continuous leaky background expression of H2B-FP progressively accumulates in quiescent HSCs and could be mistaken for permanent quiescence.
Credit: Morcos et al., 2020

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DISTINGUISHING PHYSIOLOGIC AND ONCOGENIC WNT SIGNALS

Profiling of isogenic human colon organoids reveals differences between oncogenic Wnt activation and normal Wnt signaling essential for stem cell maintenance

Around 80% of colorectal cancers (CRCs) carry truncating mutations in *Adenoma polyposis coli* (*APC*). Loss of the APC protein stabilizes the transcriptional coactivator β-catenin, a key component of the Wnt signaling pathway, leading to constitutive Wnt signaling and the formation of benign adenomas that can progress into CRC upon the acquisition of further mutations. CRC cells rely on Wnt signaling to continue proliferating but, because Wnt activity also regulates the proliferation and renewal of intestinal stem cells critical for maintaining gut homeostasis, inhibiting this pathway has highly toxic side effects in patients.

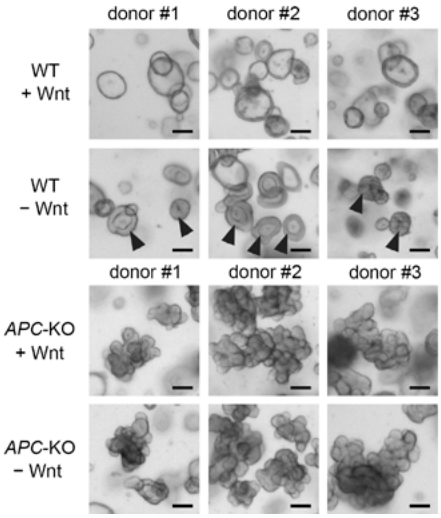
Developing successful therapies might therefore require identifying differences between normal Wnt signaling and the constitutive activation induced by oncogenic mutations in *APC* or other genes. "We set out to catalog the physiological and oncogenic Wnt responses in primary human colon epithelial cells on the transcriptome and proteome level," says Henner Farin from the German Cancer Consortium and Georg-Speyer-Haus, Goethe University Frankfurt.

Farin and colleagues, including co-first authors Birgitta Michels and Mohammed Mosa, analyzed isogenic colon organoids grown from intestinal cells isolated from healthy human subjects. RNA sequencing and quantitative mass spectrometry revealed that oncogenic Wnt signaling (induced by the CRISPR/Cas9-mediated truncation of *APC*) and physiological signaling (induced by exogenous Wnt ligands) upregulate distinct sets of genes and proteins, no matter how strongly the two signaling pathways are activated.

Many of the proteins specifically upregulated by physiological Wnt signaling are expressed in intestinal crypts, suggesting that they could be new markers for normal intestinal stem cells. On the other hand, many of the proteins specifically upregulated in response to oncogenic Wnt signaling are only expressed in tumors, and could therefore represent new biomarkers or even therapeutic targets.

Some studies have shown that high Wnt activity is associated with favorable outcomes in CRC, whereas other studies have suggested that the presence of Wnt-active stem cells correlates with increased tumor invasiveness and higher rates of relapse. "We therefore wanted to test if our organoid-derived signatures are associated with distinct clinical outcomes in CRC," says Farin.

Farin and colleagues found that the oncogenic Wnt signature was associated with good prognosis in the canonical CRC subtype CMS2, consistent with the role of *APC* mutations in this subtype and possibly reflecting an increased resemblance to benign adenomas. In contrast, the physiological Wnt signature predicted poor outcomes in CMS4 CRC, possibly reflecting tumors with increased stemness and invasiveness.



To distinguish physiological and oncogenic Wnt signaling, Farin and colleagues profiled isogenic pairs of colon organoids, cultured in the presence or absence of Wnt ligands, and carrying either wild-type or truncated versions of the *APC* gene.
Credit: Michels et al., 2019

"Oncogenic and physiological Wnt responses therefore represent two independent and opposing prognostic determinants in CRC," Farin says. "Rather than Wnt activity per se, stratification of specific downstream responses may be more informative on the status of tumors."

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Photo © Georg-Speyer-Haus

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LEUKEMIA HIJACKS MYELOID REGENERATION PATHWAYS

Correcting aberrant Notch and Wnt signaling in leukemic stem cells might normalize myeloid cell production in a number of different leukemias

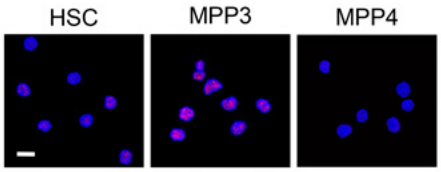
In 2015, Emmanuelle Passegué and colleagues described how hematopoietic stem cells (HSCs) maintain blood production at steady state by giving rise to a mix of multipotent progenitors (MPPs). Some, known as MPP2 and MPP3, are biased toward producing myeloid lineage cells such as macrophages and neutrophils, while others, known as MPP4, are biased toward lymphoid lineage cells like T and B cells. They also showed that in times of stress, (following HSC transplantation, for example), HSCs regenerate the blood system by inducing emergency myelopoiesis pathways that generate more MPP2 and MPP3 cells than normal, and reprogram MPP4 cells to produce more myeloid lineage cells.

Together with Yoon-A Kang and Eric Pietras, Passegué wondered whether this myeloid regeneration pathway might be constitutively activated in myeloid leukemias. "Using several different mouse models, we found that MPP3 expansion and myeloid reprogramming of MPP4 are common features of myeloid leukemia and reflect the hijacking of a normally transiently activated pathway of emergency myelopoiesis," Passegué says.

The molecular mechanisms regulating the production of different MPPs from HSCs are unknown but are likely to involve the Notch and Wnt signaling pathways. Passegué and colleagues determined that, at steady state, Notch activity is high and Wnt activity is low in HSCs and MPP4s. MPP3s, in contrast, show low Notch and high Wnt activity. Accordingly, inhibiting Notch or boosting Wnt signaling triggered the early stages of myeloid regeneration by promoting the production of MPP3s from HSCs.

Myeloid leukemias are driven by leukemic stem cells (LSCs) that are often resistant to treatment and can promote tumor recurrence. Passegué and colleagues found that Notch activity is reduced and Wnt signaling is elevated in LSCs in mice, further supporting the idea that myeloid leukemias hijack myeloid regeneration pathways and providing therapeutic targets. "We found that increasing Notch activity or decreasing Wnt activity in mouse LSCs has very similar effects in correcting myeloid cell production, delaying disease progression, and improving overall survival," Passegué says.

Notably, similar attempts to increase Notch or decrease Wnt activity in normal HSCs had no effect, with compensatory crosstalk between the two signaling pathways appearing to prevent abnormal MPP function and blood production. "Our results identify a common mechanism for myeloid leukemia development that could provide new treatment options to limit aberrant myeloid cell production," Passegué says. "This is particularly relevant for patients with no identified driver mutations or who relapsed with resistance mutations. The next step will be to identify druggable targets that could appropriately increase Notch activity while limiting Wnt activity, providing a specific anti-LSC therapy that could block their enhanced and overly biased myeloid differentiation potential."



Staining for nuclear β-catenin (red) shows that activity of the Wnt signaling pathway is high in myeloid-biased MPP3 cells but low in normal HSCs and lymphoid-biased MPP4 cells.
Credit: Kang et al., 2020

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