The identification of adult stem cells is challenging because of the heterogeneity and plasticity of stem cells in different organs. Within the same tissue, stem cells may be highly proliferative, or maintained in a quiescent state and only to be activated after tissue damage. Although various stem cell markers have been successfully identified, there is no universal stem cell marker, which is exclusively expressed in all stem cells. Here, we discuss the roles of master developmental regulator RUNX1 in stem cells and the development of a 270 base pair fragment of the Runx1 enhancer (eR1) for use as stem cell marker. Using eR1 to identify stem cells offers a distinct advantage over gene promoters, which might not be expressed exclusively in stem cells. Moreover, RUNX1 has been strongly implicated in various cancer types, such as leukemia, breast, esophageal, prostate, oral, skin, and ovarian cancers—it has been suggested that RUNX1 dysfunction promotes stem cell dysfunction and proliferation. As tissue stem cells are potential candidates for cancer cells-of-origin and cancer stem cells, we will also discuss the use of eR1 to target oncogenic mutations in stem cells and to track subsequent neoplastic changes.

**Keywords:** enhancer, eR1, RUNX1, stem cells

**INTRODUCTION**

Adult stem cells are essential for tissue homeostasis and injury repair. Stem cell plasticity and decision making are proposed to be controlled by super-enhancers of genes that define cell identity (Whyte et al., 2013). In turn, super-enhancers may be regulated by pioneer factors—master transcription factors with the ability to remodel chromatin at super-enhancers, reprogram gene expression and establish new cell fate (Adam et al., 2015). Here, we review the expression profiles and roles of master transcription factor RUNX1 (Zaret and Carroll, 2011) in diverse stem cells. We highlight the development of a molecular tool, a 270 base-pair fragment of the Runx1 enhancer (eR1) (Ng et al., 2010; Nottingham et al., 2007), as a definitive marker for adult stem cells in multiple organs. As eR1 is part of a super-enhancer driving RUNX1 expression in pathological states such as leukemia (Liau et al., 2017), we will also discuss the concept of using eR1 to target oncogenic mutations to cancer initiating cells.

**OVERVIEW OF THE RUNX1 TRANSCRIPTION FACTOR**

Runt-domain transcription factors (RUNX) are master regulators of cell-fate decisions and lineage specification in metazoan development. A typical RUNX protein has a highly conserved DNA binding domain (Runt domain) at the N-terminus and a divergent C-terminus, which regulate transcriptional activity. Although the transcriptional output of RUNX, by itself, is relatively weak, RUNX proteins collaborate with a multitude of protein partners to direct cell lineage specification cell cycle dynamics and ribosomal synthesis. Because of the Runt domain, all RUNX proteins heterodimerize with cofactor CBFβ for strong binding to the DNA consensus se-
quence 5'-PyGPYGGTPy-3' (where Py indicates pyrimidine). There are three genes—RUNX1, RUNX2, and RUNX3—in the human. Mutations of RUNX genes have been identified in various human diseases. In particular, the frequent dysregulation of RUNX genes in cancer indicates prominent roles for all RUNX genes in cancer pathogenesis (Ito et al., 2015). RUNX genes play dual roles in tumorigenesis, and can be strongly tumor suppressive or oncogenic, depending on cell context (Blyth et al., 2005; Ito et al., 2015).

RUNX1 (also known as AML1) was first discovered through a t(8;21) translocation in acute myeloid leukemia (AML) (Miyoshi et al., 1991). The RUNX1 gene spans ~261 kb (Sood et al., 2017). Two non-redundant promoters differentially regulate the expression of three major RUNX1 isoforms. The distal promoter P1 drives the expression of longest isoform RUNX1c, while P2 regulates the shortest isoform RUNX1a and the most common isoform RUNX1b (Ghozi et al., 1996). RUNX1c is mainly expressed in hematopoietic stem cells (HSCs) in the fetal liver, and T- and B-cells. RUNX1b is expressed in myeloid and other non-hematopoietic cells. Both P1 and P2 promoters are active when definitive hematopoietic cells emerge, with P2 promoter being relatively more active (Bee et al., 2010; Komeno et al., 2014; Sroczynska et al., 2009).

Runx1 in Hematopoiesis

RUNX1 is best known as the master regulator of blood development. Definitive HSCs—from which adult hematopoiesis originates—first emerge in the main arteries of the mouse embryo (de Bruijn and Dzierzak, 2017). During embryonic development, Runx1 is necessary for endothelial-to-hematopoietic transition, playing a critical role in the conversion of endothelial cells to HSC and progenitor cells. Runx1 expression in the endothelial cells of the embryonic arteries is regulated in a spatiotemporal manner and the absence of Runx1 is associated with absence of vascular hematopoietic cell clusters or HSCs (de Bruijn and Dzierzak, 2017). Runx1 has the capability to reshape the chromatin landscape by induction of histone acetylation and is associated with the recruitment of lineage specific transcription factors Tal1 and Fil1 to genomic regions proximal to Runx1-bound sites (Lichtinger et al., 2012). Moreover, RUNX1 is one of the seven transcription factors (ERG, HOXA5, HOXA9, HOXA10, LCOR, RUNX1, and SPI1) that can convert hemogenic endothelium into hematopoietic stem and progenitor cells (Sugimura et al., 2017).

During adulthood, Runx1 is expressed in most blood cells (including HSCs and progenitor cells). Mx1-Cre conditional knockout of Runx1 in bone marrow resulted in increased hematopoietic stem/progenitor cell (HSPC) pool as well as a block in development of megakaryocyte, T- and B-lymphocytes (Growney et al., 2005; Ichikawa et al., 2004). Therefore, Runx1 is required for megakaryocyte maturation and T- and B-lymphocyte differentiation. The HSPC expansion is transient and eventually leads to stem cell exhaustion. This result suggests that Runx1 is critical for maintenance of HSCs during adult hematopoiesis (Growney et al., 2005; Ichikawa et al., 2004). Runx1 haploinsufficient mice (Runx1+/-) showed a 50% reduction in long-term repopulating HSCs (LT-HSCs) and an increase in more mature, multi-lineage progenitors (Sun and Downing, 2004).

Point mutations in the Runt domain or chromosomal translocations involving RUNX1 have been identified in AML and myelodysplastic syndrome (Osato, 2004). It is proposed that these inactivating mutations of RUNX1 caused blocks in differentiation, thereby contributing to carcinogenesis. Conversely, various studies have indicated that RUNX1 might serve an oncogenic role in T-cell acute lymphoblastic leukemia (T-ALL) (Kwiatkowski et al., 2014; Sanda et al., 2012). Leukemia has been proposed to be a stem cell disorder, where aberrant differentiation blocks promote proliferation of stem cells. It would appear that leukemia may stem, in part, from RUNX1 dysfunction in HSCs.

Runx1 in Adult Hair Follicle Stem Cells

During skin development, Runx1 is expressed in a spatiotemporal manner to modulate adult hair follicle stem cell (HFSC) activation (Osorio et al., 2008). During anagen (proliferation phase), Runx1 is expressed in the bulge, outer root sheath, matrix and cortex. During catagen (regression phase), Runx1 is expressed in the lower outer root sheath. The highest levels of Runx1 are detected in the germ and lower bulge, the region which undergoes proliferation first during the telogen-anagen transition. Keratin 14 (K14)-Cre conditional knockout of Runx1 in the skin epithelial cells of adult mice resulted in impairment of hair shaft production as well as that of all temporary hair cell lineages—likely the consequences of a prolonged quiescent phase of the hair cycle and impair-ment of HFSC proliferation/colony formation (Osorio et al., 2008). The stem cell quiescence caused by Runx1 disruption was reversed by skin injury, indicating that Runx1 modulates developmental, and not injury-driven, HFSC activation. Runx1 is therefore likely to regulate early stem/progenitor cell fate choice and differentiation of hair cell lineages during development. Lineage tracing experiments in adult mice revealed not only Runx1 expression in adult long-term self-renewing and differentiating HFSCs, but also oral epithelium stem cells (Scheitz et al., 2012). Interestingly, it was proposed that Runx1 expression in the HFSCs is analogous to that of the intestinal stem cells. In the intestine, Runx1 expression is detected in villus cells and at the base of the crypts, where intestinal stem cells are located. When Lgr5CreER-IRES-GFP mice were examined, Runx1 expression partially overlapped with that of GFP (i.e., Lgr5 expressing cells), indicating that these rare Runx1-Lgr5 co-expressing cells at the crypt may be stem cells, reminiscent of Runx1 expression in the hair bulge. Moreover, Runx1 is strongly expressed in the transit amplifying cells in the upper crypt—reminiscent of the activated hair germ (Scheitz et al., 2012).

In the mouse, Runx1-expressing stem cells have been detected at the origin of skin tumors, and indeed, Runx1 was shown to be required for tumor initiation (Scheitz et al., 2012). Moreover, RUNX1 is upregulated in various human epithelial cancer types (e.g., cervical carcinoma, colon adenocarcinoma, lung and breast cancer) (Scheitz et al., 2012). In particular, Runx1 is necessary for proliferation and survival of human skin squamous cell carcinoma, oral squamous cell
cancer as well as some ovarian cancers (Scheitz et al., 2012). Depletion of RUNX1 by siRNA treatment in skin and head and neck squamous carcinoma cell lines resulted in growth inhibition, indicating that RUNX1 is essential for tumor maintenance of these cell types. Using K14-CreERT2;LSL-KrasG12D;RUNX1 KO and wildtype mice, Scheitz et al. (2012) showed that upon oncogenic KrasG12D induction, RUNX1 KO mice showed significant delays in oral and anal tumor formation, relative to their RUNX1 wildtype counterpart, which exhibited rapid formation of oral and anal tumors. It is therefore likely that aberrant upregulation of Runx1 in epithelial stem cells promote tumor initiation. Mechanistically, Runx1 stimulates STAT3 signaling through repression of SOCS3 and SOCS4 transcription to drive cancer cell growth (Scheitz et al., 2012).

**Runx1 ENHANCER ELEMENT AS A TOOL FOR IDENTIFICATION AND GENETIC MANIPULATION OF ADULT STEM CELLS**

The above studies show that RUNX1 is expressed in stem cells of multiple tissues and that deregulation of RUNX1 expression in stem cells might initiate or promote tumorigenesis. At present, how RUNX1 expression is regulated in stem cells is unclear, but it is reasonable to explore the super-enhancer concept, which has been proposed to control stem cell properties (Adam et al., 2015). From the enhancer controlling Runx1 transcription, we developed a powerful tool to specifically regulate gene expression in tissue stem cells. eR1 (also known as +23 or +24 conserved non-coding element, depending on start site definition) is a 270 base pair enhancer element that is located between the P1 and P2 promoters of the Runx1 gene (Ng et al., 2010; Nottingham et al., 2007). eR1 was identified by comparative genomic alignment, because of its high conservation across various eukaryotic species, as well as through DNaseI hypersensitivity and retroviral integration site mapping, due to its open chromatin conformation in Runx1-expressing cells (Ng et al., 2010; Nottingham et al., 2007).

**eR1 ACTIVITY DURING HEMATOPOIESIS**

We and others earlier showed that eR1 is responsible for the activation of Runx1 expression in HSPCs (Ng et al., 2010; Nottingham et al., 2007). Adult transgenic mice harboring an EGFP reporter construct with eR1 linked to the mouse heatshock protein 68 minimal promoter revealed preferential eR1 driven EGFP expression in HSCs, in agreement with strong Runx1 activity. Mouse LT-HSCs exhibited high EGFP levels while short-term HSCs (ST-HSCs) and progenitor cells showed relatively reduced EGFP expression (Ng et al., 2010). However, myeloid and lymphoid cells that express Runx1 did not show eR1 activity, suggesting that while eR1 acts as an enhancer for Runx1 expression in HSC, its activity do not reflect the global expression of Runx1 in the adult hematopoietic compartment (Ng et al., 2010).

It is unclear what regulates eR1 activity. The eR1 sequence contains conserved binding motifs for hematopoiesis-associated transcription factors such as Gata, Ets, Myb, and Runx. Work on transgenic mouse embryos indicate that transcription factors Gata2 and Ets are important for eR1 function. Moreover, the SCL/Lmo2/Ldb-1 complex was reported to occupy the eR1 site in vivo (Nottingham et al., 2007). LIM only protein 2 (LMO2) is a key regulator of HSC development, functioning as a scaffold for the multi-protein transcription factor complex, such as SCL/TAL1, the zinc finger protein GATA-1, and LIM-domain interacting protein LDB1. During zebrafish embryogenesis, transient Notch activation resulted in Runx1-dependent expansion of HSCs, suggesting that Notch and Runx1 cooperate to amplify stem cell population (Burns et al., 2005). While it is unclear whether Notch activation is directly responsible for Runx1 transcription, a recent study using a human transgenic reporter embryonic stem cell line, which harbors the Runx1 +23 enhancer linked to an eGFP reporter, indicates that Notch activation coincides with activation of the +23 enhancer. Following differentiation of the transgenic reporter cell line to hemogenic endothelial cells, only hemogenic endothelial cells which express the Notch ligand DLL4, exhibit +23 enhancer activity (Uenishi et al., 2018).

**eR1 ACTIVITY IN STOMACH STEM CELLS**

*In situ* hybridization revealed the expression of Runx1 mRNA in the upper and lower parts of the corpus gastric unit of the mouse. Moreover, immunofluorescence staining with a Runx1-specific antibody revealed the presence of Runx1 proteins in epithelial cells at the upper part of the mouse stomach corpus, and less frequently at the base of the gastric unit (Matsuo et al., 2017). Interestingly, Runx1 is co-expressed with the majority of rapidly proliferating stem cells (labelled by Ki67) at the isthmus. Aside from the corpus, Runx1 is also expressed in epithelial cells near the bottom of the pyloric gland, where Lgr5+ reserve stem cells reside (Matsuo et al., 2017). Usui et al. (2006) studied the expression of RUNX1 in stomach cancer: RUNX1 was expressed in all 11 gastric cancer cell lines and 29 gastric cancer tissues studied: no RUNX1 mutation was found in the 44 patients studied. More recently, RUNX1 was reported to play a major role in the maintenance/proliferation of gastric cancer cells through regulation of the ErbB2/HER2 signaling pathway (Mitsuda et al., 2018).

We found that eR1 directs expression to tissue stem cells of the mouse stomach corpus and antrum (Matsuo et al., 2017). Using transgenic mice harboring eR1-EGFP, we detected EGFP+ cells in the corpus epithelium. EGFP+ cells were located in the isthmus/pit region (83%), neck (7%), and base (10%) of the gastric unit. Importantly, the majority of the EGFP+ cells co-localized with Ki67 at the isthmus region, where proliferating stem cells reside. As a note of caution, EGFP/eR1 expression co-localized with only a subpopulation of Runx1+ cells, with some EGFP+ cells expressing low levels of Runx1—it would appear that the role of eR1 is to specifically upregulate Runx1 in stem cells and cannot be used to indicate Runx1 levels in all cell types. We also assessed whether eR1+ cells are involved in tissue regeneration. Following treatment of eR1-EGFP mice with tamoxifen, which induces parietal cell loss, we found that the number of EGFP+ cells per gastric unit was markedly higher in tamoxifen-treated tis-
sue than in untreated tissue, suggesting that eR1+ cells possess stem cell properties, which enable a major role in tissue regeneration. Lineage tracing in the corpus gastric unit using transgenic mice carrying eR1-CreERT2(5-2);Rosa26-LoxP-Stop-LoxP (LSL)-tdTomato to label eR1+ cells and their progeny with tdTomato revealed that eR1+ cells indeed have the regenerative capability to differentiate into all cells of the gastric unit and can therefore be defined as isthmus stem cells of the corpus. As for the 10% of eR1+ cells that were located at the base, and which co-express pepsinogen, lineage-tracing revealed that they possess tissue regeneration capacity with ribbon-like signals observed after 1-year post-injury. Organoid forming ability is inherent property of stem cells. The eR1+/tdTomato+ cells generated tdTomato+ organoids in response to Wnt signaling (i.e., Wnt3a, EGF, Noggin, R-spondin) as well as Notch-signaling (i.e., EGF, Noggin, Jagged1).

eR1 AS A TOOL TO IDENTIFY CANCER CELL-OF-ORIGIN

Together with the initial finding of eR1 as a marker of HSC, the ability of eR1 to mark stomach tissue stem cells suggest its potential use as a stem cell marker for diverse tissue types. Recently, we observed that eR1 also drives Runx1 expression in stem/progenitor cells of multiple organs, such as intestine, lung, liver and pancreas (manuscript in preparation). eR1 can therefore be used to identify and isolate tissue stem cells from multiple organs. Moreover, eR1 can specifically introduce or knockout cancer genes in stem cells. Similar to normal stem cells, tumor initiating cells and cancer stem cells are able to self-renew. Identification and targeted therapy of cancer cells-of-origin and cancer stem cells are key goals of cancer research. The activity of the RUNX1 super-enhancer, which encompasses eR1, has been implicated in T-ALL. Transcription inhibition by CDK7 inhibitor THz1 was associated with significant decreases in RUNX1 expression, as well as growth inhibition of cancer cells (Kwiatkowski et al., 2014). There is a possibility that eR1 marks cancer initiating or cancer stem cells. Indeed, the use of eR1 to specifically express oncogenic Kras in isthmus stem cells in the mouse stomach resulted in metastatic lesions reminiscent of human gastric atrophy (Matsuo et al., 2017). The cell of origin of cancer in the stomach corpus is hotly debated. Both Lgr5+ chief cells (reserve stem cells) and Mist1+ isthmus stem cells have been proposed to be cells of origin of stomach cancer (Choi et al., 2016; Leushacke et al., 2017). Our work with eR1 revealed yet another layer to the complexity of tumor formation in the stomach.

CONCLUSION

RUNX1 is increasingly shown to play essential roles in the regulation of stem cells and proliferation of various cancer types. However, how RUNX1 is activated in stem cells and how its dysregulation leads to inappropriate stem cell fate decisions remain unclear. Current data suggest that aberrant RUNX1 overexpression promote carcinogenesis at the stem cell stage. eR1 offers clues as to how RUNX1 is regulated in stem cells and upregulated in cancer cells at the super-enhancer level. We further predict that understanding the regulation of eR1 will shed light on how other pioneer factors are uniquely activated in stem cells and perhaps cancer stem cells.

The use of eR1 to specifically direct expression in stem cells of multiple organs offers an alternative to promoter driven systems because of its specific activity in stem cells and potentially, cancer cell-of-origin. In time, we will explore the use of eR1 for targeted cancer therapy at the stem cell level and cancer origin.

Disclosure

The authors have no potential conflicts of interest to disclose.

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