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New Insights Into the Role of Runx1 in Epithelial Stem Cell Biology and Pathology

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ABSTRACT

The transcription factor Runx1 has been studied in leukemia and blood for decades, but recently it has been also implicated in epithelial biology and pathology. Particularly in mouse skin Runx1 modulates Wnt signaling levels thereby regulating timely induction of hair follicle specification, proper maturation of the emerging adult hair follicle stem cells in embryogenesis, and timely stem cell (SC) activation during adult homeostasis. Moreover, Runx1 acts as a tumor promoter in mouse skin squamous tumor formation and maintenance, likely by repressing p21 and promoting Stat3 activation. Similarly, Runx1 is essential for oral epithelium tumorigenesis mediated in mice by Ras, and for growth of three kinds of human epithelial cancer cells. In contrast, Runx1 has a tumor suppressor function in the mouse intestine and shows tumor subtype specific behavior in human breast cancer. Multiple studies revealed Runx1 SNPs to be associated with human cancers and autoimmune disease. With this information as background, the field is poised for functional and mechanistic studies to elucidate the role of Runx1 in formation and/or progression of epithelial-based human disease. J. Cell. Biochem. 114: 985–993, 2013.

KEY WORDS: Runx1; HAIR FOLLICLE STEM CELLS; ORAL EPITHELIUM STEM CELLS; SQUAMOUS CELL CARCINOMA; ENDOMETROID CARCINOMA; BREAST CANCER; INTESTINAL CANCER

More than two decades ago the gene *Runx 1* (*AML1*) was commonly found at chromosome translocation points in acute myeloid leukemia (AML) [Miyoshi et al., 1991]. Since then its requirement for adult hematopoietic stem cell emergence and fate specification, and its potential role in leukemia formation drove sustained efforts to characterize its structure and function. Moreover Runx1 plays a role in neural progenitor cell proliferation. Details on Runx1 function in the hematopoietic system, leukemia, and nervous system development have been discussed elsewhere and are beyond the scope of this review [Chen et al., 2009; Kanaykina et al., 2010; Mangan and Speck, 2011]. This prospect focuses on recent findings pertaining to Runx1 function and molecular interactions in epithelial tissue homeostasis and disease biology, particularly skin, oral, and intestinal tissues.

All Runx family proteins (Runx1, Runx2, Runx3) contain the characteristic DNA binding *runt* domain that was first identified in *Drosophila* [reviewed by Friedman, 2009]. Runx1 forms a complex with the core binding factor β (CBFβ) resulting in a stable *runt* domain conformation and thus DNA binding [Yan et al., 2004]. Additionally, *Runx1* has transcriptional activation and inhibitory domains allowing it to bind to a plethora of co-factors, such as the co-repressor mSin3A and the potent co-activator p300, modulating its gene expression regulatory effects [reviewed by Friedman, 2009].

Runx1 has two promoters driving the expression of three isoforms [Ghozi et al., 1996]: the distal promoter P1 and the proximal promoter P2. P1 controls the longest isoform Runx1c. Runx1a is the shortest and Runx1b the most expressed form, and the proximal P2 promoter drives expression of both of them. All Runx1 isoforms are expressed in the hematopoietic system at different stages of development [Challen and Goodell, 2010], but little is known about their expression in other tissues. Notably, Runx1a lacks the protein binding domains but binds DNA strongly and thus potentially acts as an inhibitor to Runx1b and c. In particular it has been implicated in affecting differentiation of neural crest SCs [Kanaykina et al., 2010]. To date there are no data on Runx1a in the skin, but in keratinocytes Runx1b, but not Runx1c, is detected [Ortt et al., 2008]. Accordingly, Runx1b is strongly expressed in FACS isolated hair follicle stem cells (HFSCs), but not other basal layer skin cells [Osorio et al., 2008]. For the remainder of this review, we will consider Runx1 in general, without respect to the isoforms.

In this prospect, we first summarize Runx1's role in mouse and human HF development and homeostasis, followed by its implication as a stem cell gene in the skin and oral tissue possibly in the intestine. Second, we focus on Runx1's implications in epithelial disease, considering autoimmune disorders and various epithelial cancers, ranging from skin to endometrial cancers.

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Runx1 IN HAIR FOLLICLE STEM CELLS

HAIR CYCLE DEVELOPMENT AND HOMEOSTASIS

The hair follicle (HF) is the best-studied system for Runx1's role in epithelial tissue SCs. The HF morphogenesis occurs in pre- and post-natal life and is comprised of a permanent and a temporary portion. The permanent portion contains the bulge (Bu), which harbors the stem cells, the sebaceous gland (SG) that produces the oil necessary to lubricate the hair canal, and the infundibulum (Inf) through which the hair shafts exits the skin epidermal surface. The bulge stem cells are part of the outer root sheath (ORS) and are marked by surface expression of CD34 [Morris et al., 2004].

Hair formation starts at around embryonic day (E) 14.5 and proceeds through the developmental stages of placode, germ, peg (Fig. 1), and bulbous peg. Then the full HF develops by growing downward into the dermis and generating the inner, terminally differentiated layers of HFs: inner root sheath (IRS) and hair shaft [Schneider et al., 2009]. Starting at about postnatal day (PD) 17, morphogenesis is followed by 3-week long hair cycles spaced by more and more prolonged periods of quiescence. During each cycle, the HF undergoes three stages of remodeling. Catagen, the regression phase, characterized by apoptosis of the bulb is followed by the quiescent phase (telogen) [Schneider et al., 2009]. This is succeeded by anagen, the growth phase. At the telogen to anagen transition some HFSCs leave the bulge to differentiate and proliferate and form the matrix (M), a population of short-lived transit amplifying progenitor cells that give rise to all the differentiated, inner layers of the HFs throughout anagen. During early anagen, the bulge cells divide on average three times without further migration and replenish the HFSC pool [Waghmare et al., 2008; Zhang et al., 2009]. The synchrony of HF cycle phases and hair morphogenetic stages, and the relative abundance of HFSCs that can be isolated from the skin, makes the HF an ideal system to study tissue stem cell biology in vivo [Tumbar et al., 2004]. The HF served as a platform to begin elucidating the role of Runx1 in epithelial stem cells and cancer.

Runx1 PLAYS DISTINCT A ROLE IN HAIR FOLLICLE EPITHELIUM AND IN SKIN DERMIS

Runx1 expression in the HF was first identified by Levanon et al. [2001] using E15.5 whisker follicles of reporter mice containing LacZ inserted in frame in the Runx1 genomic locus (Fig. 1). Closer inspection of pelage HFs at E12.5 revealed the first, rare Runx1 expressing cells in the developing epidermis [Osorio et al., 2011]. At placode formation (E14.5) rare epithelial X-gal labeled cells are located in the center of emerging placode structures, and remain present in the upper region during the hair germ stage. As HF structures advance to pegs, more labeled cells are observed mostly in the center of follicles, probably corresponding to the preliminary differentiated HF lineages (cortex, IRS) [Raveh et al., 2006; Osorio et al., 2011]. At later stage pegs, Runx1 is also strongly expressed in the infundibulum protruding into the epidermis [Raveh et al., 2006; Osorio et al., 2008, 2011]. Remarkably, the Runx1 positive epithelial cells in hair placodes contribute to all layers of HF during morphogenesis and adult homeostasis, and thus these cells are precursors of adults HFSCs [Osorio et al., 2011]. On the other hand, most epithelial Runx1 expressing cells of later developmental stages (germ, bulbous peg) are short-lived and contribute less often to adult HFs [Osorio et al., 2011].

In telogen, Runx1 is expressed weakly in the lower bulge and the hair germ, the cells that proliferate first upon telogen–anagen transition [Raveh et al., 2006; Osorio et al., 2008]. Runx1 expression is extended to include the top of the bulge, and the ORS in early anagen. During full anagen, the inner differentiated cortex and cuticle layers express Runx1 in addition to the lower bulge cells and the ORS. In catagen, Runx1 is not observed in the bulge but in the ORS below it, reaching its peak during apotosis and remaining relatively high in the secondary germ [Raveh et al., 2006; Osorio et al., 2008]. Similar to HF precursor cells, the adult bulge cells expressing Runx1 are HFSCs since they contribute long-term to HF homeostasis as well as to oncogenic and physical injury repair [Scheitz et al., 2012]. In an initial study, induced epithelial knockout of Runx1 was only found to affect hair structure causing the mouse

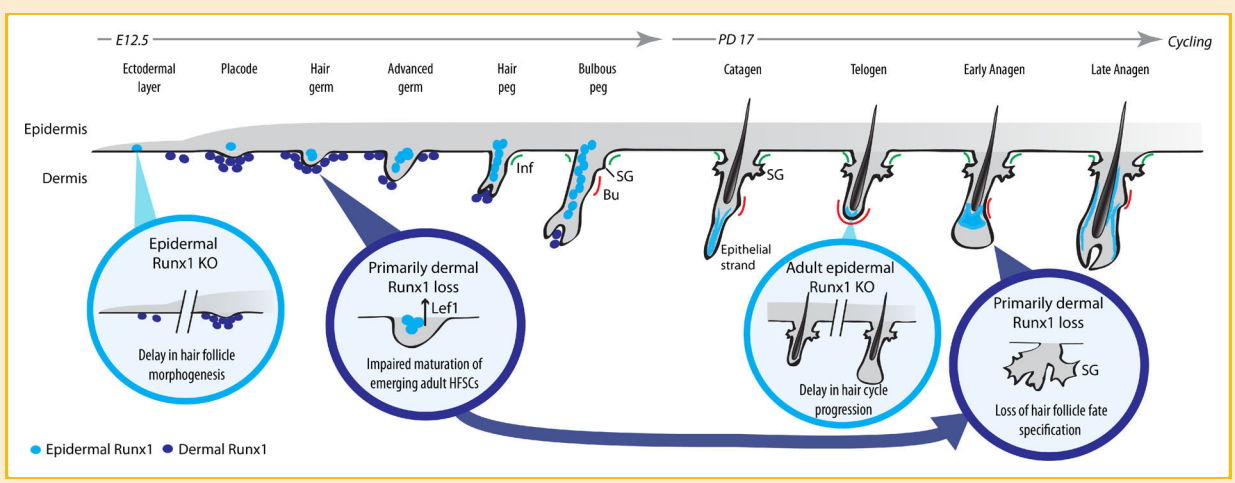

Fig. 1. Summary of the Runx1 expression pattern during hair follicle morphogenesis and the hair cycle. Bubbles summarize the effect of Runx1 loss in specific layers and at specific stages.

pelage to look ruffled, a phenotype that can be attributed to Runx1 expression in the cortex [Raveh et al., 2006]. Under closer inspection, however, Runx1 loss during morphogenesis and during adulthood causes a temporary delay in morphogenesis and hair cycle at the telogen to anagen transition [Osorio et al., 2008, 2011]. This is eventually overcome through injuries or with age [Osorio et al., 2008; Hoi et al., 2010], but it cannot be ruled out that spontaneous hair cycle progression is caused by micro injuries obtained from normal activities such as grooming. Nonetheless it is clear that both HF establishment and cycling rely on SC migration and proliferation. Given that Runx1 is expressed in SCs during both stages, a failure to initiate migration and/or proliferation in Runx1 knockout skin could explain the observed phenotypes. This hypothesis is supported by the fact that Runx1 expression precedes proliferation in bulge and germ cells of adults HFs [Osorio et al., 2008] and in vitro Runx1 is required for keratinocytes proliferation and migration [Osorio et al., 2008, 2011; Hoi et al., 2010].

The fact that injury overcomes the roadblock caused by Runx1 loss implies that different pathways are required for injury response compared to HF homeostasis and HFSC proliferation. While the homeostasis trigger is specifically localized to the HFSCs, injury causes a broad proliferation response in the IFE and the HFs including surprisingly a robust Runx1 expression [Scheitz et al., 2012].

Remarkably, mesenchymal Runx1 loss during HF morphogenesis has a much more profound effect on HF integrity than epithelial loss has. Runx1-expressing cells emerge in the dermal layer directly underlying the epidermis starting at E14.5. This dermal population increases by E17.5 and forms clusters under the hair placode, hair germ, and in the dermal condensates of emerging HFs. By birth this dermal expression diminishes drastically and by the beginning of the adult hair cycle is completely lost [Raveh et al., 2006; Osorio et al., 2008]. Using $Runx1^{CreER/fl}$ mice, Osorio et al. [2011] knocked out Runx1 at E12.5–14.5. Runx1-CreER activation showed low efficiency in the epidermis but strong efficiency in the dermis. Since these mice are not viable, Osorio et al. [2011] showed through skin grafting that in the embryonic mesenchyme Runx1 regulates cell fate specification in the adult HF even after normal protein expression has ceased. The HFs form normally in morphogenesis but in the first hair cycle, when adult stem cells generate the differentiated hair lineages, they are converted to enormous sebaceous cysts. Notably, in wild-type mice Runx1 expression in the dermis has long been lost at this point. This short, strong and specific expression of Runx1 in the dermis during embryogenesis has a long-lasting, irreversible, and profound effect on adult HF structure, in what appears as discussed later to be maturation of the emerging adult HFSCs.

Overall, Runx1's phenotypic reach in the epidermis is narrow and temporally confined within close distance of its expression. On the other hand, dermal Runx1 is far-reaching, outlasting and drastically expanding beyond its expression. But while we have made good progress in understanding the role of Runx1in HF development and homeostasis, we only begin to understand the mechanisms involved downstream and Runx1's place in the network of HF factors.

Runx1 IS PART OF A NETWORK OF FACTORS REGULATING HAIR FOLLICLE FATES

Protein interactions and signaling in the HF are complex [Lee and Tumbar, 2012] and thus the list of potential partners for Runx1 is long. To date Runx1 appears to interact with Lef1 and the Wnt signaling pathway, as well as p63 and Stat3.

The first indication that Lef1 and Runx1 might interact in hair cycle regulation was revealed using a micro-array study of wax depilated hair cycle induction. mRNAs of both Runx1 and Lef1 are up-regulated in late anagen and binding sites for both are found in genes highly expressed in the hair cortex [Ishimatsu-Tsuji et al., 2005]. Co-incidentally, epidermal Runx1 knockout skin displayed reduced Lef1 protein levels in both epidermis and dermis and reduced Wnt signaling. Specifically, mRNAs of Wnt activators were up-regulated while mRNAs of Wnt inhibitors were down-regulated by the Runx1 loss. While it is not clear if Runx1 works directly on the promoters of these genes, Runx1's role as a context-dependent transcriptional activator or repressor [Friedman, 2009] could explain this effect. These Wnt regulatory genes included secreted molecules that could explain the paracrine effect of Runx1 loss in the epidermis. Since Lef1 is a not only a mediator but also a direct target of the Wnt pathway [Lee and Tumbar, 2012], it is not surprising that its levels are regulated by Runx1 action on the Wnt pathway. Strikingly, Wnt signaling and Lef1 levels in both HF layers and dermis that are not expressing Runx1 [Osorio et al., 2011] were up-regulated when Runx1 loss was targeted mainly to the dermis via the Runx1-CreER. The opposite effect of Runx1 loss in the epidermis versus dermis on Lef1 and Wnt signaling underscores the context-dependent action of Runx1. Lef1 elevated levels persists into adulthood, a stage when Runx1 expression in the mesenchyme has already been lost in wild-type animals [Osorio et al., 2011]. This causes the misspecification of HFSCs fate to SG, as discussed in the previous section and described by Merrill et al. [2001].

p63 is so far the only identified upstream regulator of Runx1 in the HF. Ortt et al. [2008] convincingly show that intronic ΔNp63, but not TAp63, binding to *Runx1* promoter positively regulates Runx1 expression, specifically the *Runx1b* isoform. Most likely both proteins are already co-expressed at E14.5 in the developing epithelium. At this stage, Ortt et al. [2008] show broad ΔNp63 expression but fail to see Runx1 in the epithelium; later on Osorio et al. [2011] showed Runx1 to be in fact present in the HF rudiments. In adult mouse HFs Runx1 and p63 partially overlap during anagen in the lower ORS and the differentiating matrix cells. To clarify the functional significance of this regulation, site-directed mutagenesis at the binding site in keratinocytes could yield further insights. Additionally, experiments are required to analyze if the direct regulation of Runx1 by ΔNp63 is conserved across tissues and/or species because previous screens in transformed human cells lines did not uncover this relationship, which could indicate that this mechanism is specific to mice or to skin.

Lastly, Stat3 loss has a remarkably similar phenotype as Runx1 loss [Sano et al., 1999, 2000]. While the HFs develops normally, hair cycling and wound healing are impaired in Stat3 KO mice [Sano et al., 1999]. In Runx1 KO HFs Stat3 is up-regulated, possibly compensating for Runx1 loss and thus suggesting that Runx1 is found up-stream of Stat3 [Osorio et al., 2008]. Indeed Scheitz et al.

[2012] show in keratinocytes and skin cancer cells that Runx1 is essential for maintaining active Stat3 by repressing cytokine-signaling suppressors (SOCS). Strikingly, both Runx1 and Stat3 affect normal hair cycling [Sano et al., 2000; Osorio et al., 2008], however, externally induced hair cycling appears to function independently from both factors. This strongly suggests that the mechanism for Runx1-dependent Stat3-regulation discovered in vitro translates to in vivo HF biology.

It is likely that in the future we will be able to identify more partners of Runx1 in this network that is regulating HF biology.

Runx1 IN HUMAN HAIR

All the findings acquired in mice begged the question of the role of Runx1 in normal and diseased human skin. Importantly, when Runx1 expression was compared in mice and men similar patterns emerged in the HF at different hair cycle stages [Raveh et al., 2006; Soma et al., 2006; Osorio et al., 2008; Hoi et al., 2010]. Human Runx1 expression partially overlaps with that of human keratin associated protein 5 (hKAP5.1). In accordance with the presence of putative Runx1 binding regions in the promoter of hKAP5.1, human cultured keratinocytes show increased hKAP5.1 levels upon Runx1 overexpression [Soma et al., 2006]. However, more functional studies in human keratinocytes or ex vivo cultures are needed to verify protein interactions and molecular functions of candidate factors for human HF homeostasis. In the case of Runx1 it appears that at least its expression pattern is conserved from mouse to humans, giving us hope that this will also be true for its functional consequences.

Runx1 IN OTHER EPITHELIAL TISSUE STEM CELLS

Runx1 expression has been observed in other stratified epithelial organs similar to skin such as nails and keratinized pads [Raveh et al., 2006], but its potential role or overlap with putative SCs other than HF and blood has been unclear. Recent evidence suggests that Runx1 may be present in other epithelial SCs. Scheitz et al. [2012] show that Runx1 expressing cells are present in the basal layer of the oral epithelium. Lineage tracing of these cells in Runx1CreER;td-Tomato mice shows that some cells remain in the basal layer for at least 18.5 weeks and give rise to the differentiated epithelium [Scheitz et al., 2012], hence fulfilling the stem cell definition of long-term self-renewal and differentiation. Moreover, Runx1 was detected in some LGR5+ cells of the intestinal SC region and also showed high levels in the differentiated epithelial cells of the crypt, a pattern that resembles that observed in the HF [Osorio et al., 2008; Scheitz et al., 2012]. Runx1 promoter activity is also found at the base of colon crypts [Scheitz et al., 2012]. Fijneman et al. [2012] present some evidence that in the absence of Runx1 expression of SC specific genes increases, supporting a putative regulatory role for Runx1 in the colon SCs. However, conclusive lineage tracing data to demonstrate the lineage potential of Runx1 expressing cells in the intestine and colon awaits generation of a Runx1-CreER knockin mouse line that can efficiently be activated for recombination in these tissue [Scheitz et al., 2012]. On the whole, we are just beginning to discover and understand Runx1 biology in tissue function and maintenance.

Runx1 IN AUTOIMMUNE DISEASES

Due to its widespread activation upon injury Runx1's role in the skin may well extend beyond HF growth regulation [Osorio et al., 2008; Scheitz et al., 2012]. Indeed SNP-association studies have identified Runx1 as a potential candidate in several autoimmune diseases, which commonly show skin symptoms (summarized in Table I). It is interesting to note that these associations originate from SNPs within Runx1 binding sites, which may prevent Runx1 from

TABLE I. Reported Runx1 Involvement in Cancer Formation and Autoimmune Diseases

Disease type	Study type	Refs.
Cancer		
Skin SCC	Conditional mouse KO model	Hoi et al. [2010]
Skin SCC	Inducible mouse KO model Tumor lineage tracing Mouse and human in vitro culture Human tissue staining and microarray meta-analysis	Scheitz et al. [2012]
Head and neck SCC	Human in vitro culture Human tissue staining and microarray meta-analysis	Scheitz et al. [2012]
Endometroid carcinoma	Human tumor tissue staining Human tumor microarray data	Planagumà et al. [2004, 2006, 2011]
Triple negative breast cancer	Human in vitro culture Human tumor microarray data	Wang et al., 2011
PR+ breast cancer	Human tumor genome sequencing	Banerji et al. [2012]
Prostate cancer	Human primary cancer vs. normal microarray	Yeh et al. [2009]
Prostate cancer	Human SNP association study	Huang et al. [2011]
Intestinal cancer	Inducible mouse KO model Mouse KO vs. WT tissue microarray	Fijneman et al. [2012]
Colon and rectal cancer	Human SNP association study	Slattery et al. [2011]
Colorectal cancer	Human cancer vs. normal microarray meta-analysis	Pradhan et al. [2012]
Autoimmune disease		
Psoriasis	Human SNP association study Human tissue staining	Helms et al. [2003]
SLE	Human SNP association study Human tissue expression assays	Prokunina et al. [2002]
Rheumatoid arthritis	Human SNP association study Human tissue expression assays	Tokuhiro et al. [2003]

regulating expression of its target gene. Systemic lupus erythematosus (SLE) was the first autoimmune disease with a suggested Runx1 function. A study by Prokunina et al. [2002] identified an intronic SNP in PDCD1 (programmed cell death 1) associated with SLE that disrupts a Runx1 binding site within an enhancer element. Although the directionality of the effect could not be established due to large patient variation, the authors showed that with this intronic SNP PDCD1 fails to bind Runx1 resulting in altered PDCD1 expression [Prokunina et al., 2002]. No further functional studies were done, but it is known that PDCD1 regulates T-cell function and regulates self-antigen tolerance [Fife and Pauken, 2011] and thus the axis of Runx1-PDCD1 is a good candidate for autoimmune disease. Rheumatoid arthritis is the only autoimmune disease where functionally independent SNPs within *Runx1* and its binding sites have been identified [Tokuhiro et al., 2003]. A SNP within a Runx1 binding site in SLC22A4, a membrane-transporter, is associated with rheumatoid arthritis and causes increased suppression of SLC22A4 due to increased Runx1 binding [Tokuhiro et al., 2003]. Expression of SLC22A4 is limited to the hematological and immunological tissues but the functional consequences of its mis-expression are not known.

While both SLE and rheumatoid arthritis have distinct skin phenotypes, patients are affected primarily by other problems, such as joint pain. Psoriasis, on the other hand, is a skin specific inflammatory disease featuring increased dermal vasculature, T-cell invasion, and overall immune-mediated keratinocyte hyper proliferation [Griffi and Barker, 2007]. A family based study by Helms et al. [2003] identified a causative SNP close to the sodium/hydrogen exchanger SLC9A3R1 that disrupts a Runx1 binding site causing loss of Runx1 binding and ceased *SLC9A3R1* transcriptional activity. Given their observation that *SLC9A3R1* is expressed strongly in inactive but not active T-cells, the authors speculate that loss of Runx1 binding causes T-cell activation through a lack of SLC9A3R1. The fact that disease-associated SNPs have not been identified in Runx1 itself indicates that Runx1 must remain functional, while its target SLC9A3R1 does not. Overall, it seems plausible that SLC9A3R1 is regulated by Runx1 because in addition to the SNP-associated site this gene locus contains 32 predicted Runx1 binding sites in humans, 18 of which are conserved to Rhesus macaque and 3 to Mouse.

Functional studies have not been performed yet for many of these SNP associations, but they serve as a solid basis for future investigation of Runx1 role in human disease. To take our knowledge to the next level we need to derive functional connections with biological significance. In this respect, we know most about Runx1 role in the field of cancer biology. Below we summarize SNP associations and cancer-related functional biological studies in human and in model organisms.

Runx1 IN SOLID TUMORS

BIOLOGICAL AND MECHANISTIC EVIDENCE IN SKIN CANCER

Because of its context-dependent function in cell proliferation, apoptosis, and cell differentiation, Runx1 may act in different epithelial cells and tissues as either a tumor suppressor or as an oncogene. Whereas a tumor suppressor function has been suggested recently in intestinal cancer [Fijneman et al., 2012], the first evidence of an oncogenic function is derived from fibroblasts where Runx1 overexpression causes oncogenic transformation [Kurokawa et al., 1996]. This is in line with the observed block of proliferation in keratinocytes upon Runx1 loss [Osorio et al., 2008] and to date there is significant evidence that Runx1 is required for tumor formation in the skin. Human skin squamous cell carcinoma (SCC) over-expresses Runx1 and loss of this protein prevents SCC formation in mouse skin [Hoi et al., 2010; Scheitz et al., 2012]. In fact, Runx1 is required for tumor initiation in the HFSCs and based on lineage tracing the majority of papilloma (benign squamous tumors which are precursors of SCC in mice) originate from Runx1 expressing HFSCs [Scheitz et al., 2012]. Runx1 is crucial for proliferation in normal keratinocytes, while during tumor growth loss of Runx1 can be temporarily overcome with the help of other proliferative agents, such as 12-O-tetradecanoylphorbol-13-acetate (TPA) [Scheitz et al., 2012]. More importantly, Runx1 loss in fully developed mouse tumors leads to significant tumor regression and similarly primary human skin SCC cell lines cannot survive without Runx1 [Scheitz et al., 2012]. Hence, Runx1 is required for tumor and cancer cell growth and survival and is a prime potential target for SCC prevention and treatment. Although these data clearly show the dependence of tumors on Runx1, an oncogenic function in the skin has not been clearly demonstrated. In fact, ectopic expression of Runx1 in non-stem cells during injury is insufficient to render those cells tumorigenic, attesting to the importance of the epigenetic context to the Runx1 role in cancer.

Scheitz et al. [2012] also extended the skin phenotype to head and neck SCC, which are suppressed in mice in the absence of Runx1. Moreover, oral human SCCs cell lines require Runx1 for their growth. In the future, Runx1 contribution to esophageal, cervical and lung SCC development and maintenance should be explored, since Runx1 is expressed there at high levels according to Oncomine data [Scheitz et al., 2012]. However, even at the current state, the remedy of a Runx1 inhibitor in skin SCC treatment cannot be ignored. SCC is the second most common cancer affecting 0.8 million new patients annually in the US alone [Alam and Ratner, 2001]. Current treatment is often disfiguring since SCCs occur frequently on the face and require lengthy and expensive surgery. A non-invasive treatment that could be applied by the patient himself would not only benefit the patient but also reduce health care costs.

Mechanistically, Runx1 regulates several genes that have been shown to influence cancer formation in various tissues, namely p21 and Stat3 (Fig. 2). p21 is significantly up-regulated in the HFSCs upon Runx1 loss [Osorio et al., 2008] and double knockout of Runx1 and p21 in mouse keratinocytes rescues cell proliferation in vitro [Hoi et al., 2010]. Hence, Runx1 may mediate its tumor promoting phenotype partially through repressing p21, already expressed in the cells of tumor origin. Additionally, Runx1 prevents expression of the suppressors of cytokine signaling 3 and 4 (SOCS3 and 4) by binding to their promoters, allowing for activation of Stat3 by phosphorylation through the Jak/Stat pathway [Scheitz et al., 2012]. The combination of Stat3 activation and p21 repression makes Runx1 a central player in SCC formation. Both genes are involved in other cancers and it remains to be seen if Runx1 is up-stream in those cases as well. Additionally, this connection brings up the

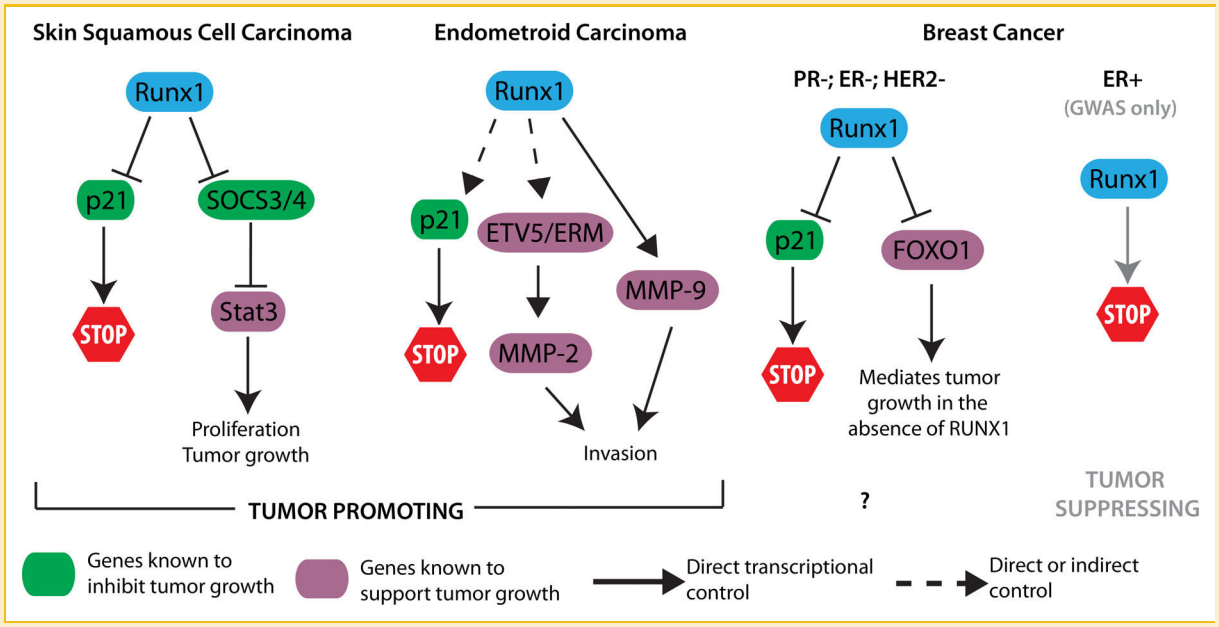

Fig. 2. Known pathways of Runx1's function in three cancer types.

question if Runx1 is a broader cancer regulator than previously envisioned. In the following section we will review the evidence that has been collected in other epithelial tissues to give cues for future cancer research.

THE ROLE OF Runx1 IN OTHER SOLID TUMORS

Similar to the findings in autoimmune diseases Runx1 has been associated with various cancers in SNP studies. In prostate cancer SNPs within *Runx1* are associated with an increased risk of cancer progression and lymph node metastasis leading to a worse patient prognosis [Huang et al., 2011]. Additionally, Runx1 expression increases with pathological stage [Yeh et al., 2009] implying that Runx1 has an oncogenic function and that the SNPs boost or misdirect its activity. In particular, SNPs in Runx1 also correlated with SNPs in Stat3; Yeh et al. [2009] suggest that in this context both genes regulate expression of EZH2. Although these findings were not tested yet in vivo, they provide some intriguing additional evidence that Runx1 can affect tumor development through Stat3.

Colon and rectal cancer were significantly associated with tagging SNPs for Runx1 in a study of two Caucasian case–control cohorts [Slattery et al., 2011]. Similar to previous studies of these cancers, single SNPs do not withstand stringent statistical testing due to small effect sizes. However, Slattery et al. [2011] show that simultaneous occurrence of SNPs in *eIF43*, *Runx1*, and *Runx3* represents a significant combined risk for colon and rectal cancer. Similarly, combinations of *Runx1* SNPs were associated with CIMP+, MSI+, Kras2, and TP53 colon cancer subtypes and its interactions with SNPs in Smad3, Smad7, BMPR1B, BMPR1A, and TGFβR1 strongly increases the risk for colon and rectal cancer. This study does not conduct biological experiments to identify the functional consequences of these SNPs, yet it suggests that Runx1's phenotype is the result of complex network interactions. At the same time a computational study of cancer microarrays has identified Runx1 positively associated with colon cancer development [Pradhan et al., 2012]. Strikingly, a study in mice gives reason to assume that Runx1 can also act as a suppressor of intestinal cancers. Both wild-type and Apc^{Min} mice develop significantly more colon and small intestine tumors in the absence of Runx1 [Fijneman et al., 2012]. Notably, both skin SCC and intestinal cancers have been shown to originate from the tissue SCs [Barker et al., 2009; Lapouge et al., 2011; White et al., 2011; Scheitz et al., 2012]. Thus Runx1 loss is affecting both SC populations. Still it seems that at least in mice its effect on tumor formation lies on opposite ends of the spectrum. In humans Runx1 seems to be strongly over-expressed in SCCs and colon cancers supporting an oncogenic function, while in intestinal cancers Oncomine analysis uncovered Runx1 as neither up- nor down-regulated [Scheitz et al., 2012]. At this point, it is not clear if the tumor-suppressor function is conserved across species or intestinal cancer subtypes.

Microarray and immunofluorescence data on primary human tumors, along with loss of Runx1 function studies on cultured cancer cells suggest that at least head and neck and skin SCCs have a conserved Runx1 function, but in breast cancer there is more than one mechanism at play. While in triple negative breast cancer (ER−; PR−; HER2−) Runx1 was either present or compensated by FOXO1 up-regulation [Wang et al., 2011], in oestrogen-receptor-positive (ER+) breast carcinomas Runx1 (or CBFβ) is lost in 4 out of 37 cases [Banerji et al., 2012] (Fig. 2). Breast epithelial cells (MCF10A) have a molecular expression profile similar to triple negative cancers. Within this in vitro model genes have multiple verified Runx1 binding sites, among them FOXO1, p21, and p57. In this system, Runx1 binds and down-regulates FOXO1 and p21 [Wang et al., 2011 and Fig. 2]. These and other genes show a heterogeneous expression pattern that is dependent on phosphorylated Runx1 [Wang et al., 2011]. This evidence points out that post-translational modification is essential for Runx1 function in these cells, a connection that

already has been made for hematopoiesis [most recently, Yoshimi et al., 2012]. Notably, the down-regulation of p21 by Runx1 is similar to that observed in the skin [Hoi et al., 2010]. Therefore, one might expect MCF10A cells without Runx1 to fail to grow too, but this did not occur. Breast cancer cell lines continue to grow and in MCF10A cells Runx1 loss even leads to hyper-proliferation that is strictly dependent on FOXO1 up-regulation [Wang et al., 2011; Scheitz et al., 2012]. Alternatives to FOXO1 compensation mechanism may be present for some human cancer cells that express high levels of Runx1, while some cells cannot grow in the absence of Runx1 despite high FOXO1 expression [Scheitz et al., 2012]. Runx1 presence in triple negative cancers and its suppression of p21 suggest an oncogenic function, but its connection with FOXO1 is rather tumor-suppressor like. The data on Runx1 and p21 suggest that Runx1 is tightly interwoven in this network of cell cycle regulators that inhibit cyclin dependent kinases, which in return phosphorylate Runx1 at least in breast cells [Wang et al., 2011]. This supports the notion that Runx1 is a complex regulator and there are various mechanisms by which it could act as a tumor promoter or suppressor, even in subclasses of the same cancer and it emphasizes that we still have a lot to learn about Runx1 in the different breast cancer types.

Finally, there is evidence for Runx1 involvement in endometroid carcinoma and to date the evidence suggests that similar to SCCs Runx1 function is conserved across subtypes. Similar to skin SCCs, Runx1 is expressed close to the invasive edge in endometroid carcinoma and, moreover, is required for myometrial invasion [Planagumà et al., 2004]. During cancer development Runx1 but also p21 levels increase gradually, peaking at invasion [Planagumà et al., 2006]. While the authors do not have evidence favoring a direct or indirect interaction, they propose three hypotheses, all involving stabilization of p21 levels leading to suppressed cell proliferation while inducing differentiation. Notably, p21 is more broadly expressed than Runx1 and levels correlate with proliferation in cells lacking Runx1 [Planagumà et al., 2006]. Hence, it is possible that in endometroid cancer Runx1 expression mediates a switch from a proliferative to an invasive phenotype, matching the Runx1 expression pattern peaking at myometrial invasion.

In addition to p21, Runx1 expression shows a correlation with MMP-2 and -9 [Planagumà et al., 2011 and Fig. 2], two gelatinases that are key to degrading the basement membrane. In both endometroid and ovarian carcinoma the MMPs and Runx1 are expressed most strongly at the invasive edge during the stage of invasion. These data support a hypothesis in which Runx1 mediates myometrial invasion through blocking proliferation and inducing membrane degradation. Although Planagumà et al. [2011] do not analyze if Runx1 binds MMP-2 and -9 promoters directly, it has been shown previously that Runx1 and Runx2, bind to and cause promoter activation of MMP-9 in bone metastatic cancer cells [Pratap et al., 2005]. They also show that Runx2 regulates bone cancer cell invasion, and its overexpression causes increased invasion potential in metastatic (MDA-MB-231) and non-metastatic (MCF-7) breast cancer cell lines. It is possible that Runx1 also mediates bone cancer invasion given the overlap of the evidence in endometroid cancer and the MMP-9 promoter binding bone cancer, a possibility that should be evaluated further.

Lastly, Planagumà et al. [2011] also uncover a strong correlation of Runx1 expression at the proliferative edge with the expression of transcription factor ETV5/ERM in both ovarian and endometroid cancer. ETV5/ERM directly binds the promoter and regulates MMP-2 expression [Monge et al., 2007]. Hence it would be plausible that Runx1 activates MMP-9 and ETV5/ERM transcription, which in turn activates MMP-2 to lead to myometrial infiltration. Interestingly, expression of ETV5/ERM has also been connected to malignancy of esophageal SCC and other malignancies [Yuen et al., 2011].

It is striking how many cancers appear to be characterized by regulatory networks involving Runx1 and how fast this list has grown in the recent years. It remains to be seen, if more cancers will be added to this list and how Runx1 function in normal homeostasis relates to its role in cancer formation, progression, and metastasis for each tissue type.

CONCLUSION

The common theme emerging for Runx1 function across tissues is its implication in stem cell regulation and in cancer. In addition to hematopoietic stem cells and neural progenitors [Kanaykina et al., 2010] in the last few years Runx1 has been reportedly expressed in three other stem cell populations: HF, oral epithelium, and potentially in the intestine. Strong functional and genetic studies implicate Runx1 as a key regulator of both HSCs and HFSCs function. It will be interesting to examine how global Runx1 function is as a regulator of other adult tissue stem cells and cancers. Association and functional preliminary data in breast, colon, intestinal, and prostate suggest compelling directions for future studies. Moreover, the link between immune diseases and malfunctions of Runx1 requires further in depth investigation.

Runx1 roles in HSCs and HFSCs overlap to some extent but are distinct. In both systems early Runx1 expressing cells mark SC precursors and are required for adult tissue maintenance. Additionally, Runx1 is also expressed in the short-lived progenitors that form the first or "primitive" wave of hair and hematopoietic lineages, respectively. Next, Runx1 signaling is essential to control proper adult SC activation and differentiation and overall Runx1 loss leads to a lack of differentiated blood and hair lineages. Naturally, there are differences in the details of these processes. Most importantly, impediment of HFSC activation in adulthood and timely emergence of hair placodes (which include the precursors of adult HFSCs) due to Runx1 loss is reversible. However, hematopoietic SCs cannot recover from Runx1 loss, since fetuses die prematurely due to secondary effects [Chen et al., 2009]. Moreover, loss of Runx1 in the environment did not have an effect on the proper emergence of adult HSCs [Chen et al., 2009], while the adult HFSCs showed severe defects in maturation that became manifested by adulthood [Osorio et al., 2011]. By focusing our work on new tissues, we can begin to understand the capacity of Runx1 as a potential conductor of the stem cell concert that is performed daily in our bodies.

While there seem to be many common themes of Runx1 function in tissue stem cells, the opposite seems to be true for cancer development. So far we see three independent mechanisms through

which Runx1 can regulate cancer development. First, Runx1 represses p21 expression and thus positively regulates cell cycle progression in several tissues. Additionally, Runx1 is required for Stat3 phosphorylation, supporting cellular proliferation. Lastly, Runx1 binds and activates MMPs to potentially facilitate invasion. Notably, Runx1 modulates Wnt signaling in normal skin biology and it is possible that this connection applies to cancer, since Wnt activation is a major path to cancer promotion [Polakis, 2012]. Each of these mechanisms alone is associated with cancer formation and together they indicate that most frequently Runx1 acts as a tumor promoter in epithelial tissues. Whether these mechanisms are mutually exclusive or may occur in the same tumor type remains to be seen. Further research is required to connect the branches of this cancer-regulating network with Runx1 as a central player. However, we have to be careful not to limit ourselves to a tumor-promoting function, since compelling evidence in mice showed that Runx1 can act as a tumor suppressor in some epithelial tissues. By all means, we need to keep an open mind in further exploring this remarkable protein as it turns out clearly that Runx1 is not a one trick pony!

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