Molecules and Cells

Minireview

RUNX1 Dosage in Development and Cancer

Michael Lie-a-ling[1,6], Renaud Mevel[1,6], Rahima Patel[1], Karen Blyth[2,3], Esther Baena[4], Valerie Kouskoff[5,*], and Georges Lacaud[1,*]

[1]Cancer Research UK Stem Cell Biology Group, Cancer Research UK Manchester Institute, The University of Manchester, Macclesfield, SK10 4TG, UK, [2]Cancer Research UK Beatson Institute, Glasgow, G61 1BD, UK, [3]Institute of Cancer Sciences, University of Glasgow, Glasgow, G61 1QH, UK, [4]Cancer Research UK Prostate Oncobiology Group, Cancer Research UK Manchester Institute, The University of Manchester, Macclesfield, SK10 4TG, UK, [5]Division of Developmental Biology & Medicine, The University of Manchester, Manchester, M13 9PT, UK, [6]These authors contributed equally to this work.
*Correspondence: georges.lacaud@cruk.manchester.ac.uk (GL); valerie.kouskoff@manchester.ac.uk (VK)
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The transcription factor RUNX1 first came to prominence due to its involvement in the t(8;21) translocation in acute myeloid leukemia (AML). Since this discovery, RUNX1 has been shown to play important roles not only in leukemia but also in the ontogeny of the normal hematopoietic system. Although it is currently still challenging to fully assess the different parameters regulating RUNX1 dosage, it has become clear that the dose of RUNX1 can greatly affect both leukemia and normal hematopoietic development. It is also becoming evident that varying levels of RUNX1 expression can be used as markers of tumor progression not only in the hematopoietic system, but also in non-hematopoietic cancers. Here, we provide an overview of the current knowledge of the effects of RUNX1 dosage in normal development of both hematopoietic and epithelial tissues and their associated cancers.

Keywords: development, dosage, hematopoiesis, runx1, tumorigenesis

INTRODUCTION

RUNX1 is the founding member of the mammalian core-binding transcription factor family which also consists of RUNX2, RUNX3 and their non-DNA binding co-factor core-binding factor beta (CBFβ) (Ito et al., 2015; Mevel et al., 2019). In humans, *RUNX1* is localized on chromosome 21 and was first identified by Miyoshi et al. (1991) as the acute myeloid leukemia gene 1 (*AML1*) due to its involvement in the t(8;21) translocation in acute myeloid leukemia (AML). Shortly after this discovery, the murine version of *Runx1* was identified (Bae et al., 1993; Ogawa et al., 1993b; Wang et al., 1993) which paved the way for the development of *Runx1* knockout mouse models. These models revealed that RUNX1 plays a crucial role in the establishment of the hematopoietic system during embryogenesis (North et al., 1999; Okuda et al., 1996; Wang et al., 1996a). Both in ontogeny and disease, there are indications that the dose of wild-type (WT) RUNX1 can have profound effects on cell survival and differentiation. Although arguably best studied in hematopoiesis and leukemia, RUNX1 has also been found to play important roles in the development and tumorigenesis of epithelial tissues (Hong et al., 2019; Mevel et al., 2019; Taniuchi et al., 2012). Here, we aim to provide an overview of the current knowledge of the effects of RUNX1 dosage, in mouse and human, during normal development and homeostasis of hematopoietic and epithelial tissues as well as the known requirements for endogenous WT RUNX1 in cancers.

Dosage reflects both the amount of protein as well as its activation status. Indeed, RUNX1 protein levels can be regulated by the rate of transcription, translation and stability.

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RUNX1 activity (either as activator or repressor) is also further modulated through protein conformation, intracellular localization, post-translational modifications (PTMs) and interactions with additional proteins. RUNX1 has many interacting partners and their availability depends on cell type, differentiation status and cell cycle. Describing these interactors fully lies outside the scope of this brief review and has been covered in great details in excellent recent reviews (Chuang et al., 2013; Goyama et al., 2015; Ito et al., 2015). In vertebrates, there is a high degree of homology between the different RUNX proteins both within and across species (Rennert et al., 2003). However this high degree of inter-gene similarity does not necessarily mean that mechanisms of action and regulation of RUNX1 can be extrapolated to other RUNX family members (Bruno et al., 2019). This review focuses specifically on what is known about RUNX1 in human and mouse.

RUNX1 PROTEIN LEVELS AND RUNX1 ACTIVITY

Two promoters control *RUNX1* transcription, the P1 (distal) and the P2 (proximal) promoter, whose major generated transcripts are respectively the distal *RUNX1c* and the proximal *RUNX1b* isoforms (Ghozi et al., 1996; Miyoshi et al., 1995). The two promoters are differentially active depending on the cell context and developmental stage (Bee et al., 2009; Draper et al., 2016; Sroczynska et al., 2009). P1 transcripts are longer than P2 transcripts due to the presence of a 150 kb intron suggesting that the former takes longer to produce (Levanon et al., 2001; Pozner et al., 2000). Furthermore, both isoforms possess different 5' and 3' untranslated regions containing motifs known to affect post-transcriptional events like RNA stability and the rate of translation initiation (Levanon et al., 2001; Levanon and Groner, 2004; Pozner et al., 2000). At the protein level, the two isoforms only differ in their most N-terminal amino acid sequence (Fig. 1A). The unique N-terminus of RUNX1b has been implicated in protein stability (Nieke et al., 2017), while the unique N-terminal sequence of RUNX1c has been shown to have higher binding capacity on certain genes (Telfer and Rothenberg, 2001). The common regions of both isoforms consist of a N-terminal region, which potentially plays a role in transcriptional activation (Liu et al., 2006), followed by the DNA binding Runt homology domain which also forms the interaction domain for the RUNX family co-factor CBFβ. CBFβ is the heterodimeric binding partner of all RUNX proteins (Nagata et al., 1999; Ogawa et al., 1993a; 1993b). CBFβ enhances RUNX DNA-binding affinity and protects it from degradation (Bravo et al., 2001; Huang et al., 2001; Tahirov et al., 2001; Yan et al., 2004). Interestingly, two different isoforms of CBFβ have been described which, at least in the case of RUNX2, have been shown to differentially affect DNA binding (Jiang et al., 2016). The C-terminal half of RUNX1 harbors a transactivation domain, flanked by inhibitory regions (Aronson et al., 1997; Kanno et al., 1998; Levanon et al., 1998). In primates there is a third commonly expressed RUNX1 isoform, *RUNX1a*, transcribed from the P2 promoter (Miyoshi et al., 1995). This isoform lacks most of the C-terminus including the transactivation domain. In mice, it is thought that an exon 6 skipping variant of *Runx1b* is fulfilling a similar role (Komeno et al., 2014).

Finally, RUNX1 activity and stability can be modulated by various PTMs including phosphorylation, methylation, acetylation, ubiquitination, sumoylation and prolyl isomerisation (Blumenthal et al., 2017; Goyama et al., 2015; Ito et al., 2015). In Table 1, we have listed the residues in RUNX1 that have been shown to be the target of PTM and their effect on RUNX1. Few of these PTM have been extensively studied *in vivo* neither in development nor in cancer models. In general multiple residues have to be mutated to see clear phenotypes *in vivo* suggesting, perhaps not unexpectedly, that there is a high degree of redundancy and/or compensation in place (Goyama et al., 2004; Huang et al., 2012; Tachibana et al., 2008; Yoshimi et al., 2012).

RUNX1 IN HEMATOPOIESIS AND LEUKEMIA

RUNX1 dosage in hematopoietic development

In mammalian embryogenesis, the hematopoietic system is established via several consecutive waves of blood cell generation (Dzierzak and Bigas, 2018). In mice, the first wave generates primitive erythrocytes at embryonic day 7.25 (E7.25). It is followed by the emergence of erythroid myeloid progenitors at E8.25, and lymphoid myeloid progenitors at E9.5. The final wave of hematopoiesis at E10.5 takes place in the aorta-gonad-mesonephros (AGM) region of the embryo proper and generates the first hematopoietic stem cells (HSCs). The HSCs then migrate to the fetal liver (E12.5) where they multiply and mature before colonizing the bone marrow (E16.5). Except for the first wave, RUNX1 is absolutely required for blood cell formation (Chen et al., 2009; Lancrin et al., 2009; North et al., 1999; Okuda et al., 1996; Wang et al., 1996a; Yokomizo et al., 2008). At all sites of *de-novo* blood cell generation in the embryo, the hematopoietic cells have been found to arise from a specialized endothelium (hemogenic endothelium or HE), via a process termed the endothelial-to-hematopoietic transition (EHT) (Boisset et al., 2010; Chen et al., 2009; Eilken et al., 2009; Lancrin et al., 2009; Ottersbach, 2019; Zovein et al., 2008). RUNX1 is required for EHT (Chen et al., 2009; Lancrin et al., 2009; Liakhovitskaia et al., 2009; Menegatti et al., 2019) and there are indications that RUNX1 dosage is important for the progression and timing of this process.

Detailed studies using reporter mice and mouse embryonic stem cell lines (mESCs) demonstrated that the P2 promoter (*Runx1b* isoform) is activated first during ontogeny (Bee et al., 2009; Sroczynska et al., 2009). The mESCs system further revealed that the P2 promoter is active from the hemangioblasts (the mesodermal precursor to HE) stage onwards (Lie-A-Ling et al., 2018; Sroczynska et al., 2009). Both *in vivo* and in the mESCs system, it is clear that the P2 promoter is dominant in the HE, while afterwards, as the first hematopoietic stem and progenitor cells (HSPCs) emerge, the P1 promoter becomes active (Bee et al., 2009; Sroczynska et al., 2009). *In vivo*, upon migration of the HSPC to the fetal liver, P2 activity decreases and P1 becomes the dominant promoter (Bee et al., 2009; Sroczynska et al., 2009). Quantification of *Runx1* RNA levels in bulk sorted populations derived from mESCs suggest *Runx1* expression is higher in hematopoietic progen-

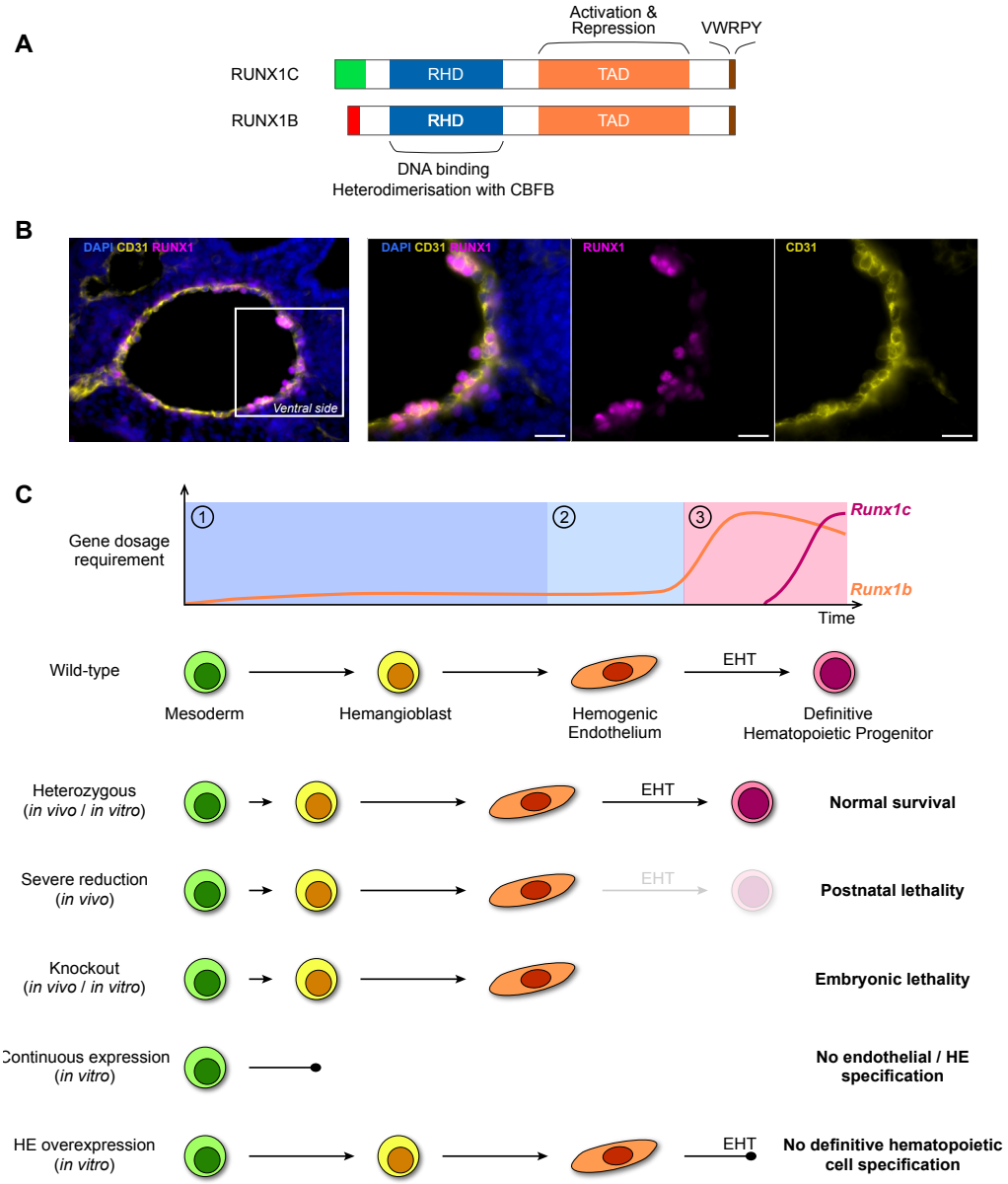

Fig. 1. RUNX1 dosage in hematopoietic development. (A) Schematic representation of the two most abundant RUNX1 isoforms. Except for the most N-terminal sequence (RUNX1C N-terminal in green, RUNX1B N-terminal in red) the proteins are identical and they both contain the highly conserved Runt homology domain (RHD, blue) followed by a transactivation domain (TAD, orange) which is flanked by inhibitory regions. The C-terminal inhibitory region contains a highly conserved VWRPY motif (brown). (B) Immunofluorescence on the AGM of a E10.5 mouse embryo. The dorsal aortic endothelial cells are marked by the endothelial marker CD31 (yellow). The majority of the cells on the ventral side of the dorsal aorta (constituting both endothelial and rare HE cells) are positive for the RUNX1 protein (magenta). Scale bars = 20 μm. (C) Current model of RUNX1 dosage in hematopoietic development. Top: RUNX1 dosage requirement can be divided in three phases. Phase ①: early in differentiation RUNX1 is not required but its (low) dose influences the timing and dynamics of HE cells appearance. Phase ②: although RUNX1 levels are still low in HE cells, its presence is required for the initiation of the EHT. Phase ③: an increased dose of RUNX1 is required for the completion of EHT and the generation of the first mature hematopoietic cells. The whole differentiation process is predominantly controlled by the RUNX1b isoform. Bottom: schematic overview of the currently available phenotypic data on RUNX1 dosage during the establishment of the hematopoietic system in the embryo.

itors than in the preceding differentiation stages including HE (Goode et al., 2016; Lie-A-Ling et al., 2018). Similar observations have been made by single cell polymerase chain reaction analyses (Swiers et al., 2013) and RNA-seq (Baron et al., 2018) of cells isolated from mouse AGM, with the frequency of *Runx1* expressing cells increasing according to differentiation stage. Despite potentially lower levels of *Runx1* expression in HE, immunofluorescence analyses of the AGM

Table 1. Post translational modifications of RUNX1

Post translational modification	Effect	Modifier	Target domain	Target residues (Runx1b)
Serine/threonine phosphorylation (1)	Increased transactivation, decreased stability	ERK	Predominatly C-term transactivaton domain	S249, S266, S276, S435, T273
Serine/threonine phosphorylation (1)	Increased transactivation, decreased stability	Hip2k	Predominatly C-term transactivaton domain	S249, S276, T273
Serine/threonine phosphorylation (1, 2)	Increased transactivation, decreased stability	CDK	Predominatly C-term transactivaton domain	S21, S249, S266, S276, S397, T273
Tyrosine phosphorylation (3)	Increased transactivation, increased stability, reduced HDAC interaction, increased DNA binding	Src kinase	Predominatly C-term inhibitory domain	Y260, Y375, Y378, Y379, Y386
Methylation (4)	Reduced SIN3a interaction, increased transactivation activity	PRMT1	C-term inhibitory domain	R2016 and R210
Methylation (5)	Reduced transactivation via increased co-repressor DPF2 binding	PRMT4	C-term transactivation domain	R223
Acetylation (6)	Reduced DNA binding, reduced transactivation	p300/CBP	N-terminus	K24, K43
Ubiquitination (7)	Increased degradation	STUB1 E3 ubiquitin ligase	Predominantly runt domain	K24, K43, K83, 90, 125, 144, 167, 182, 188 (potential targets)
SUMOylation (8)	Unknown (reduced transactivation shown for RUNX3)	PIAS1	Runt domain	K144
Prolyl isomerization (9)	Increased acetylation, stability and transactivation activity	PIN1	Not defined	Not defined

Currently described post translational modifications of RUNX1 and their effect on RUNX1. All amino acid residues are numbered based on RUNX1b. The number between brackets (#) refers to the following citations: 1, (Aikawa et al., 2006; Biggs et al., 2006; Imai et al., 2004; Tanaka et al., 1996; Wee et al., 2008; Zhang et al., 2004); 2, (Guo and Friedman, 2011); 3, (Huang et al., 2012; Leong et al., 2016); 4, (Zhao et al., 2008); 5, (Vu et al., 2013); 6, (Yamaguchi et al., 2004); 7, (Shang et al., 2009; Yonezawa et al., 2017); 8, (Kim et al., 2014); 9, (Islam et al., 2014).

in mice has demonstrated that the majority of the cells on the ventral side of the dorsal aorta (constituting both endothelial and rare HE cells) are positive for the presence of the RUNX1 protein (Fig. 1B) (North et al., 1999).

In human, the picture is less clear. Early publications indicate that during human ESCs (hESCs) differentiation the expression of *RUNX1* isoforms is similar to that of the mESCs system whereby *RUNX1b* precedes *RUNX1c* (Challen and Goodell, 2010; Ditadi et al., 2015; Ng et al., 2016), whereas recent papers report that *RUNX1c* is expressed first (Angelos et al., 2018; Navarro-Montero et al., 2017). Similar to data obtained in mice, RNA-seq on single cells from human embryos demonstrated that *RUNX1* expression can be detected in cells with arterial endothelial gene expression profiles (likely constituting both endothelium and HE), and as the cells differentiate to HSPCs, the proportion of *RUNX1* expressing cells increases (Zeng et al., 2019).

Modulation of gene dosage has been extensively used to assess the effect of RUNX1 dosage changes in ontogeny. Although total *Runx1* KO is embryonic lethal, heterozygous mice appear unaffected (North et al., 1999; Okuda et al., 1996; Wang et al., 1996a). However, closer inspection revealed profound effects on the window of HSC emergence which is expedited by approximately half a day (Cai et al., 2000; Mukouyama et al., 2000; Wang et al., 1996a; 1996b). In contrast, a more severe reduction of *Runx1* levels, by homozygous disruption of the P2 promoter, leads to postnatal death (Bee et al., 2010; Pozner et al., 2007). Potential dosage effects are also observed when the RUNX1 non-DNA binding partner *Cbfβ* is deleted (Niki et al., 1997; Sasaki et al., 1996; Wang et al., 1996b). Indeed, although *Cbfβ* knockout mice appear to phenocopy the *Runx1* KO models, generation of hypomorphic *Cbfβ* alleles resulted in a slight delay in the window of mortality when compared to the *Runx1* KO animals and the presence of a few hematopoietic progenitors in these embryos (Wang et al., 1996b). Evidence from the mESCs system, closely modeling yolk sac hematopoiesis, is in line with the data obtained *in vivo* and demonstrated that the reduction of RUNX1 through haploinsufficiency expedites blood development by 12 h (Lacaud et al., 2002; Lacaud et al., 2004). Conversely, overexpression of RUNX1 in both human and mESCs blocks hematopoiesis. In hESCs, *RUNX1* overexpression from the ESC stage onwards has no effect on mesoderm commitment but disrupts subsequent endothelial and HE specification (Chen et al., 2017). Overexpression in mESCs derived HE appears to induce an accelerated EHT without the emergence of mature hematopoietic cells, while low levels of *Runx1* can induce a productive EHT (Lie-A-Ling

et al., 2018). Furthermore, it was also demonstrated that RUNX1 is required for both the initiation and completion of EHT and that both events may require a different dose of RUNX1.

Taken together, the current data indicate that the initial establishment of the hematopoietic system relies on a low dose of RUNX1 and that careful modulation of this low dose controls the dynamic and progression of blood formation (Fig. 1C).

RUNX1 mutations and requirement in leukemia

Considering its importance in the ontogeny of the hematopoietic system, it is not surprising that *RUNX1* has been found to be a recurrent target of genomic alterations in hematological disorders (reviewed in Bellissimo and Speck, 2017; Sood et al., 2017). *RUNX1* is implicated in more than 50 chromosomal translocations leading to pediatric acute lymphoblastic leukemia (ALL), AML and myelodysplastic syndrome (MDS). In addition to translocations, mono or bi-allelic somatic mutations of *RUNX1* have been documented in MDS, AML, ALL and chronic myelomonocytic leukemia (CMML). Finally, germline mono-allelic mutations of *RUNX1* are associated with familial platelet disorder with predisposition to AML (FPD/AML).

In terms of dosage, high levels of *RUNX1* mRNA are frequently observed in AML, T cell-ALL (T-ALL) and B cell-ALL (B-ALL) (Sun et al., 2019). Increased *RUNX1* transcription is in particular observed in B-ALLs and is associated with the fusion of *ETV6* to *RUNX1* (TEL/AML1) (Gandemer et al., 2007; Robinson et al., 2003; Soulier et al., 2003). In this context, increased *RUNX1* mRNA is a positive prognostic marker although its precise role is unclear. In T-ALL, the non-mutated WT *RUNX1* allele is important for leukemogenesis and tumor survival (Choi et al., 2017). Here, RUNX1 is required for the expression of a subset of TAL-1 and Notch regulated genes, including *MYB* and *MYC*, which are required for maintenance of the leukemia. Consequently, the deletion of WT *Runx1* in a mouse T-ALL model or small molecule mediated inhibition of RUNX1 in patient samples can impair leukemic growth. Interestingly, RUNX1 inhibition did not affect normal hematopoietic cells, indicating a specific requirement for WT *RUNX1* in T-ALL cells (Choi et al., 2017).

In AMLs, increased *RUNX1* transcript levels have been associated with both, *de-novo* AMLs and AMLs harboring the *FLT3-ITD* (internal tandem duplication) (Behrens et al., 2017; Salarpour et al., 2017). In the latter case, RUNX1 cooperates with FLT3 to induce leukemia. Also, it is striking that *RUNX1* mutations appear to be absent in patients with leukemogenic fusion protein leukemias (Patel et al., 2012; Schnittger et al., 2011; Tang et al., 2009). In this context, dependency on WT *RUNX1* has been shown for AML1-ETO (t8;21), CBFB-SMMHC (inv16), MLL-AF9, and CBFB-MYH11 (inv16) translocation leukemias (Ben-Ami et al., 2013; Goyama et al., 2013; Hyde et al., 2015). In the case of AML1-ETO, WT RUNX1 and the RUNX1-ETO fusions both target many identical sites in the genome. However, binding is mutually exclusive and it is the balance between the two proteins that is driving the transcriptional networks maintaining leukemia (Ptasinska et al., 2014). Investigation of CBFB-MYH11 (inv16) has shown that leukemia containing fusion protein variants with reduced WT RUNX1 binding/inhibition are more leukemogenic than their stronger RUNX1 inhibitory counterparts (Hyde et al., 2015; Kamikubo et al., 2010). The need for the right balance between oncogenic mutation/fusion and WT RUNX1 is further highlighted by the finding that patient samples with intermediate WT *RUNX1* levels tend to have a poor prognosis (Morita et al., 2017a). Additionally, depletion of *RUNX1* has been shown to lead to compensation by the other RUNX family members *RUNX2* and *RUNX3* (Morita et al., 2017a; 2017b). The addiction of leukemia to WT *RUNX1* extends to AML expressing mutated forms of *RUNX1*, with its knockdown negatively affecting leukemic cells (Mill et al., 2019). Finally, patient studies demonstrated allelic imbalances in the transcriptional activity of mutant and WT alleles, further highlighting the potential importance of the dosage of WT RUNX1 in a leukemic context (Batcha et al., 2019).

RUNX1 IN EPITHELIAL TISSUES AND CANCERS

The role of RUNX1 dosage during the development and homeostasis of epithelial tissues remains less documented than in the hematopoietic setting. However, increasing evidence suggests a role for RUNX1 in various non-hematopoietic tissues of epithelial origin (reviewed in Mevel et al., 2019). Indeed, high throughput next-generation sequencing has revealed relatively high frequencies of genomic alterations of *RUNX1*, and *CBFβ* in solid cancers (Blyth et al., 2005; Ito et al., 2015), albeit to lower levels than in leukemia (Figs. 2A and 2B). Interestingly, while it is yet to be fully determined to what extent these alterations contribute to tumor biology, mutations of *RUNX1* have been associated with loss of function (van Bragt et al., 2014). Beyond the presence of these mutations, earlier studies identified *RUNX1* mRNA as part of a 17-gene signature associated with metastasis in a panel of adenocarcinomas, including breast and prostate cancers, with its expression inversely correlating with tumor aggressiveness (Ramaswamy et al., 2003). Overall, under- and over-expression of endogenous *RUNX1* has been found in several solid tumors, reinforcing the idea that it is broadly implicated in the biology and pathology of epithelial tissues (Blyth et al., 2005; Ito et al., 2015; Scheitz et al., 2012).

RUNX1 in hormone-related cancers

Hormone-related cancers constitute some of the most common cancers in women and men, and *RUNX1* alterations have been reported in all of these malignancies. To date, the role of RUNX1 in solid tumorigenesis has been best studied in mammary tissue (Riggio and Blyth, 2017). The normal breast epithelium is one of the few epithelial tissues for which changes in RUNX1 dosage have been reported during normal physiology/homeostasis. In addition to differential expression levels of *RUNX1* in the basal and luminal compartments of the mammary ducts, *RUNX1* levels have also been shown to fluctuate during pregnancy and lactation (Blyth et al., 2010; McDonald et al., 2014; van Bragt et al., 2014). In mice, *Runx1* was demonstrated to be a crucial regulator of the ER+ mammary luminal lineage. Deletion of *Runx1* led to a reduction of ER+ mature luminal cells, which could be rescued by the loss

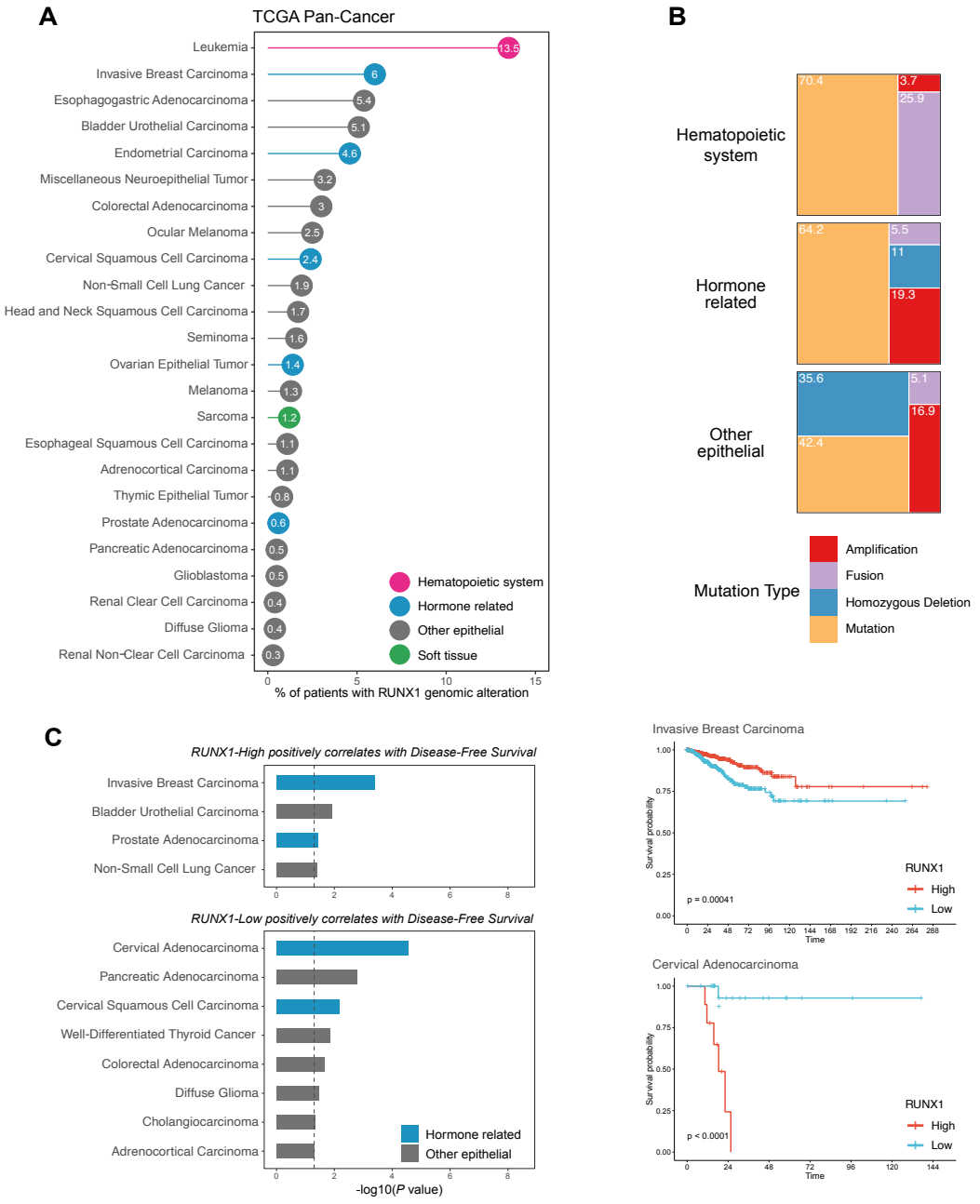

Fig. 2. Meta-analysis of *RUNX1* alterations and prognostic value in the TCGA PanCancer atlas. (A) Frequency of *RUNX1* genomic alterations across the TCGA PanCancer atlas. Cancers with no alterations were excluded. Cancers affecting the hematopoietic system are colored in pink, hormone related cancers in blue, cancers of soft tissues in green, and other epithelial cancers in grey. (B) Proportion of *RUNX1* amplification, homozygous deletion, fusion and mutation in cancers affecting the hematopoietic system, hormone related cancers, and additional epithelial cancers. Soft tissue cancers were excluded from these analyses due to the small number of patients affected. (C) Prognostic value of RUNX1 mRNA expression using the TCGA PanCancer Atlas expression data, in terms of Disease-Free Survival. Datasets of the TCGA PanCancer Atlas were downloaded from cBioPortal (https://www.cbioportal.org/). Briefly, patients were split in RUNX1-High and RUNX1-Low groups using the "surv_cutpoint" function of the "survminer" R package ("minprop" argument set to 0.1). Cancers were then separated into two groups, depending on whether RUNX1-High and RUNX1-Low groups are significantly associated with a better prognosis (P value < 0.05 using the univariate log-rank test). Representative examples of the corresponding Kaplan-Meier curves are shown for the Invasive Breast Carcinoma and Cervical Adenocarcinoma datasets (defined by the "Cancer Type" column of the TCGA PanCancer Atlas clinical data).

of either *Trp53* or *Rb1* (van Bragt et al., 2014). With regards to cancer, several studies involving large patient cohorts have identified recurrent *CBFβ* and *RUNX1* mutations (Banerji et al., 2012; Cancer Genome Atlas Network, 2012; Ellis et al., 2012; Kas et al., 2017; Nik-Zainal et al., 2016; Pereira et al., 2016). At the protein level, high-grade primary breast tumors also displayed in general reduced levels of *RUNX1* compared to low/mid-grade tumors (Kadota et al., 2010). These observations have led to the hypothesis that RUNX1 could have a tumor suppressor function. The proliferation of ER+ breast cancer cells was increased upon *RUNX1* knockdown, which led to estrogen-mediated *AXIN1* suppression and enhanced β-catenin activation (Chimge et al., 2016). In agreement with a tumor suppressor role, a link has emerged between RUNX1 and suppression of the epithelial-to-mesenchymal transition (EMT) process. Indeed, downregulation of *RUNX1* in the normal mammary epithelial cell line MCF10A was sufficient to induce hyperproliferation and abnormal morphogenesis (Wang et al., 2011). The morphological changes observed upon *RUNX1* knockdown were characteristic of an EMT, and associated with the activation of transforming growth factor β (TGFβ) and WNT signaling pathways (Hong et al., 2017). Both *RUNX1* and *RUNX3* were also shown to prevent the induction of YAP-mediated EMT in this same cell line (Kulkarni et al., 2018). Likewise, the RUNX1-CBFβ complex was able to prevent the migration potential of the ER+ breast cancer cell line MCF7 in an ER-dependent manner (Pegg et al., 2019). The emerging role of RUNX1 in EMT is not unexpected considering its well documented role in EHT, a process often referred to as 'EMT-like' (Hamidi and Sheng, 2018; Monteiro et al., 2016). However, while RUNX1 is critical for the induction of EHT during hematopoietic development, it appears to act as a gatekeeper of EMT in breast cancer cells.

In contrast to its putative tumor suppressive functions, RUNX1 is also believed to be associated with oncogenic roles. Indeed, higher *RUNX1* mRNA levels were found in the triple-negative breast cancer subgroup (Karn et al., 2011; Rody et al., 2011). This was later corroborated by a strong correlation between high RUNX1 protein levels and poor prognosis in triple-negative and ER-negative breast cancers (Ferrari et al., 2014). Increased expression of *RUNX1* was also associated with disease progression in patient samples and in the MMTV-*PyMT* mouse model. Interestingly, the invasiveness of the cells isolated from this mouse model could be repressed by knocking-down *Runx1* expression (Browne et al., 2015), suggesting that its role in EMT may be context-dependent.

Beyond breast cancer, overexpression of *RUNX1* was correlated with overexpression of p21WAF1/CIP1 in invasive endometrioid carcinoma, where it was suggested to play a role in promoting myometrial infiltration (Planaguma et al., 2004; 2006). In this respect, Doll and colleagues found that ectopic overexpression of *RUNX1* in the endometrial cancer cell line HEC1A was associated with the establishment of distant metastasis (Doll et al., 2009). High levels of *RUNX1* were also reported in human epithelial ovarian tumors, and its knockdown in the SKOV-3 cell line led to a decrease in proliferation, migration, and invasion (Keita et al., 2013).

Although less substantial than in female-related cancers, there is accumulating evidence for a potential role of RUNX1 in prostate cancer. Single-nucleotide polymorphisms within the *RUNX1* gene—such as the rs2253319 polymorphism—were associated with an increased risk of prostate cancer progression and metastasis (Huang et al., 2011). *RUNX1* was also found amplified in a significant proportion of neuroendocrine castration-resistant prostate cancer (Beltran et al., 2016). However, the biological relevance of these alterations, if any, remains unknown. Contrasting studies looking at RUNX1 expression in prostate cancer have reported that *RUNX1* mRNA increases with pathological stage (Yeh et al., 2009), while protein levels have been reported to be decreased in advanced forms of the disease (Takayama et al., 2015). Interestingly, the links between RUNX1 and hormones reported in breast cancer (Riggio and Blyth, 2017) seem to extend to the prostate gland which is particularly rich in androgens. In *Nkx3.1/Pten* mutant mice, prolonged exposure to reduced androgens levels resulted in prostate tumors with up-regulated *Runx1* (Banach-Petrosky et al., 2007). *RUNX1* has also been shown to be a downstream target of androgen receptor signaling, and is thought to play divergent roles in AR-dependent and castration-resistant prostate cancer cell lines (Takayama et al., 2015). With regards to the growing importance of stroma-cancer interactions, downregulation of *RUNX1* expression in mesenchymal stem cells was shown to reduce their proliferative potential in response to TGFβ, before their differentiation into prostate cancer-associated myofibroblasts (Kim et al., 2014).

RUNX1 in skin cancers

In keeping with its role in hematopoiesis, *Runx1* dosage has been found to be important for hair follicle stem cells. During homeostasis, reduced levels of *Runx1* favors self-renewal of bulge stem cells (Hoi et al., 2010), while high *Runx1* expression promotes differentiation into early progenitor hair germ cells (Lee et al., 2014). RUNX1 has been linked to skin cancer in mice, where its activated expression during chemically induced skin carcinogenesis was proposed to be oncogenic (Hoi et al., 2010). In line with this, loss of *RUNX1* impaired the proliferation of human oral and skin squamous cell carcinoma cell lines (Scheitz et al., 2012). *Runx1* was also found essential for the survival and proliferation of cultured keratinocytes (Hoi et al., 2010), notably by regulating fatty acid production (Jain et al., 2018).

Other tissues

RUNX1 has also been linked with tumors of the gastrointestinal tract, where it was found to be frequently downregulated (Miyagawa et al., 2006; Sakakura et al., 2005). In conditional mouse models, *Runx1* deletion is sufficient to induce intestinal tumorigenesis (Fijneman et al., 2012). In gastric cancer cell lines, both the knockdown of RUNX1 and its therapeutic inhibition resulted in reduced tumorigenic potential via suppression of the ErbB2/HER2 signaling pathway (Mitsuda et al., 2018). Finally, the previously noted emerging link between RUNX1 and EMT has also been documented in colorectal cancer (Li et al., 2019), and renal fibrosis (Zhou et al., 2018) in which RUNX1 acts as an inducer of EMT. Increased expression of *RUNX1* was also predictive of poor prognosis in patients diagnosed with clear cell renal cell carcinoma (Fu et al., 2019).

CONCLUSIONS

It is now well established that RUNX1 dosage is important during normal development and homeostasis of hematopoietic tissues, and there is a growing body of evidence indicating that it is important in epithelial tissues as well. These studies highlight the multifaceted characteristics of RUNX1, in particular in non-hematopoietic tissues, where it was not originally thought to be involved. Alterations of RUNX1 dosage in these tissues were initially revealed by large scale genomic studies and these results are reinforced by growing experimental evidence implicating RUNX1 in crucial hallmarks of cancer progression such as cell proliferation, EMT or DNA repair (Tay et al., 2018). It has now become clear that RUNX1 can act both as an oncogenic or a tumor-suppressive factor (Blyth et al., 2005; Ito et al., 2015; Neil et al., 2017). Intriguingly, the implication of RUNX1 in both female and male related cancers has revealed a close relationship with ER and AR, which warrants further investigations. While the functional evidence between RUNX1 dosage and cancer development is often still lacking and requires further work, it has become evident that varying levels of RUNX1 expression can be used as markers of tumor progression in specific clinical cohorts (Fig. 2C).

Finally, although systems modifying RUNX1 dosage via (conditional) knock-out alleles as well as controlled transcriptional regulation provide valuable information on how RUNX1 dosage can affect normal physiology and cancer, detailed stage and cell type-specific information on physiological RUNX1 dosage levels would drive our understanding even further. In this context, it should be emphasized that when evaluating RUNX1 dosage, both the amount of protein as well as its activation status should be taken into consideration. Currently, it is still very challenging to fully assess the different parameters regulating RUNX1 dosages. However, the continuous improvement of single-cell technologies might soon allow us to interrogate, at a single-cell level, the quantity and ratios of RUNX1 isoforms, as well as their PTMs. Such data would provide valuable insights on RUNX1 dosage at the single cell level and would allow us to better investigate their functions.

Disclosure

The authors have no potential conflicts of interest to disclose.

ORCID

Michael Lie-a-ling https://orcid.org/0000-0003-0194-4313
Renaud Mevel https://orcid.org/0000-0002-2742-6576
Rahima Patel https://orcid.org/0000-0002-5678-2537
Karen Blyth https://orcid.org/0000-0002-9304-439X
Esther Baena https://orcid.org/0000-0003-4157-3684
Valerie Kouskoff https://orcid.org/0000-0001-9801-4993
Georges Lacaud https://orcid.org/0000-0002-5630-2417

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