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RUNX3: A New Player in Myeloid Gene Expression and Immune Response

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Abstract RUNX transcription factors function as scaffolds for interaction with various coregulatory proteins during developmental processes such as hematopoiesis, neurogenesis, and osteogenesis. The current view places RUNX proteins within the TGF-β signaling pathway, although each one exhibits cell- and tissue-specific functions. In the case of RUNX3, recent data have suggested its function as a tumor suppressor factor and highlighted its involvement in immune cell differentiation and activation. The molecular mechanisms for the pleiotropic effects of Runx3 deficiency are not completely understood. The present article will summarize the known functional activities of RUNX3, emphasizing its role in myeloid cell gene expression and its potential contribution to the migratory and adhesive capabilities of this cell lineage. J. Cell. Biochem. 98: 744–756, 2006. © 2006 Wiley-Liss, Inc.

Key words: transcription; myeloid; dendritic cell; adhesion

RUNX proteins are context-dependent transcriptional regulators, as they activate or repress transcription depending on the DNA context surrounding their binding sites and on physical interactions with other transcriptions factors, co-activators, and co-repressors [Lutterbach and Hiebert, 2000]. Mammalian Runt-related (RUNX) transcription factors are homologous to proteins encoded by the *Drosophila* segmentation genes *runt* and *lozenge*, which are involved in sex determination, neurogenesis, and cell fate specification during hematopoiesis [Kania et al., 1990; Daga et al., 1996]. The RUNX gene family contains three members (*RUNX1*, *RUNX2*, and *RUNX3*), all of which heterodimerize with a common partner (CBF-β) [Levanon and Groner, 2004], and is defined by the presence of the Runt domain, a 128-amino-acid conserved region which mediates both sequence-specific DNA binding and heterodimerization with CBF-β [Ito, 2004].

The three proteins regulate developmental processes and might be involved in the pathogenesis of autoimmune diseases [Alarcon-Riquelme, 2003]. Although the three RUNX factors are now placed within the TGF-β signaling pathway [Miyazono et al., 2004], each one exhibits a tissue-specific pattern of expression and, consequently, modulate transcription in a tissue-specific manner. RUNX1 and RUNX2 are required for hematopoiesis [de Bruijn and Speck, 2004] and osteogenesis [Stein et al., 2004], respectively, and are genetically altered in leukemia and bone disorders. Characterization of Runx3−/− mice has evidenced that RUNX3 is involved in a large variety of physiological processes including neurogenesis, thymopoiesis, and dendritic cell functional maturation, and a large amount of information now suggests that it might be a tumor suppressor, as numerous cancers exhibit a defective expression of RUNX3. In the present review, we will primarily focus on RUNX3 and its role in myeloid gene expression, and hypothesize about its potential involvement in the acquisition of the adhesive capabilities exhibited by myeloid cells during inflammatory and immune responses.

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RUNX3 GENE AND PROTEIN STRUCTURE

The *RUNX3* gene, located on the short arm of chromosome 1 (1p36.1) and on chromosome 4 in mouse [Levanon et al., 1994], is the smallest of the three *RUNX* genes and contains six exons (Fig. 1). The *RUNX3* gene shares a similar genomic organization with the rest of mammalian *RUNX* loci, including the presence of two alternative promoters (P1, distal; P2, proximal) containing several RUNX binding sites [Rini and Calabi, 2001; Drissi et al., 2002; Levanon and Groner, 2004]. The in vitro activity of the alternative RUNX3 promoters correlates with RUNX3 cellular expression pattern, suggesting that they contribute to the cell type-restricted expression of RUNX3 [Bangsow et al., 2001]. With the exception of the CCAAT box and Sp1-binding sequences within P2, the regulatory elements and transcription factors controlling RUNX3 expression are currently unknown. Interestingly, the P2 proximal promoter is included within a CpG-rich region, which covers around 4,000 bp around the initiation ATG, and whose methylation greatly affects RUNX3 expression. Transcription from these two alternative promoters originates proteins with different N-termini (Fig. 1). In P1-derived transcripts, exon 1 codes for the N-terminal part of the protein (MASNS), exons 2–4 code for the Runt domain and exons 5 and 6 code for the C-terminal part of the protein. By contrast, P2-derived RUNX3 proteins exhibit an alternative N-terminus (MRIPV) coded for by a sequence within exon 2 (Fig. 1). In addition, the diversity of RUNX3 proteins is further increased by the fact that, like the other members of the family, isoforms are generated from the *RUNX3* gene by alternatively splicing events.

All the RUNX proteins share a highly conserved "signature" region (Runt domain, 90% identity), located between residues 54 and 182 in RUNX3, and involved in CBF-β heterodimerization and recognition and binding to the consensus DNA sequence PyGPyGGT. The stability and DNA-binding affinity of RUNX proteins is greatly increased upon heterodimerization with the CBF-β subunit, an association that protects RUNX proteins from degradation by the proteasome [Huang et al., 2001a]. The C-terminal region of the protein (182–415) is responsible for the transcriptional activity and ends with the sequence VWRPY, a recruitment motif for the Groucho/TLE family of co-repressors [Levanon et al., 1998]. RUNX proteins are focally localized within the nucleus, and their subnuclear localization requires signals for nuclear import and for targeting to the nuclear matrix [Stein et al., 2004], both of which are located downstream of the Runt domain. RUNX proteins have been shown to associate with a number of transcription factors, co-activators, and co-repressors (Smads, Ets, STAT, AP-1, Myb, C/EBP, p300, TLE), with the interacting regions mapping both to the Runt domain and the C-terminal region of the molecule [Ito, 2004]. In the case of RUNX3, the interaction

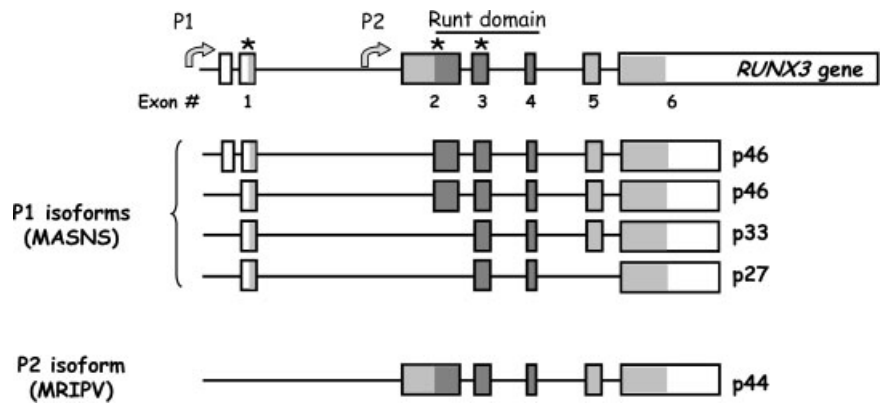

Fig. 1. Genomic organization of the human RUNX3 gene and structure of alternatively spliced RUNX3 mRNAs. Arrows indicate the position of the two alternative promoters. Boxes represent the individual exons, and shadowed areas illustrate the coding sequence. The Runt domain-encoding areas are also shown. Asterisks indicate the respective translation initiation methionines. Isoforms generated from the distal P1 promoter exhibit the N-terminal sequence MASNS. The prototypic isoform derived from the proximal P2 promoter exhibits the alternative N-terminus MRIPV.

with Smad-3 and TLE has been mapped to the C-terminus [Levanon et al., 1998; Hanai et al., 1999], whereas both the Runt domain and the C-terminal region are implicated in the association with p300 [Jin et al., 2004]. Although many target genes have been described for RUNX1 and RUNX2, only a few genes have been specifically identified as RUNX3 targets. An updated list of RUNX3 target genes is shown in Table I, which includes a significant number of genes involved in cellular adhesion and migration (see below).

RUNX3 AND ONCOGENESIS

The implication of RUNX1 in the genesis of myeloid leukemias, together with the absence of RUNX3 in 50% of AML-M2 patient blasts and the inhibition of RUNX3-dependent transactivation by AML1/ETO and TEL/AML1 [Meyers et al., 1996], has previously led to the hypothesis that RUNX3 could also be a target of oncogenic fusion proteins. However, although RUNX3 expression inversely correlates with relapse and survival rate, no evidence of mutations affecting RUNX3 has been obtained so far in acute myeloid leukemia [Otto et al., 2003], thus suggesting that RUNX1 and RUNX3 display non-redundant functions in myeloid cells. Supporting this idea, the generation of "RUNX chimaeric molecules" has shown that although the C-terminal portion of RUNX3 can functionally substitute the corresponding region of RUNX1 in the initial development of fetal liver hematopoiesis in vivo, it cannot fully replace the corresponding region of RUNX1 [Fukushima-Nakase et al., 2005].

RUNX factors function as both tumor suppressors and dominant oncogenes in a context-dependent manner [Blyth et al., 2005]. The oncogenic potential of RUNX3 has been observed in CD2-Myc transgenic mice and retrovirus-induced T-cell lymphomas [Blyth et al., 2005]. The relationship between RUNX3 loss of function and cancer was first described in Runx3-knockout mice [Li et al., 2002], which develop hyperplasia of the gastric epithelium that stems from an increased resistance to the TGF-β growth inhibitory activity and TGF-β-induced apoptosis. Furthermore, the genesis and progression of human gastric cancer correlates with decreased RUNX3 levels secondary to a combination of hemizygous deletion of the gene and hypermethylation of the CpG island in the P2 promoter [Li et al., 2002]. In fact, 60% of gastric cancer cell lines and tumor tissues exhibit P2 promoter hypermethylation, a percentage that

TABLE I. RUNX3 Target Genes

Gene	Regulated in	Up/down	Method	Reference
TCR-β	T cells	Up	T-TF	Meyers et al. [1996]
Defensin NP-3	Myeloid cells	Up	EMSA; T-TF	Westendorf et al. [1998]
Germ-line IgCα	B cells	Up	EMSA; T-TF	Hanai et al. [1999]
MDR-1	Leukemic cells; gastric cells	Down	EMSA; T-TF	Javed et al. [2000]; Guo et al. [2005]
CD4	$CD8^+$ $CD4^-$ thymocytes	Down	In vivo	Taniuchi et al. [2002]
CD11a	Myeloid cells	Up	EMSA; T-TF; ChIP	Puig-Kroger et al. [2003]
LINE-1s	B cells	Up	EMSA; T-TF	Yang et al. [2003]
CD11c	Dendritic cells	Up	In vivo; T-TF	Fainaru et al. [2004]
CD11b	Dendritic cells	Down	In vivo	Fainaru et al. [2004]
RUNX1	B cells	Down	EMSA; T-TF	Spender et al. [2005]
CD103	$CD8^+CD4^-$ thymocytes	Up	In vivo	Grueter et al. [2005]
Bcl-2	Gastric cancer cells	Down	EMSA	Guo et al. [2005]
MRP1	Gastric cancer cells	Down	EMSA; T-TF	Guo et al. [2005]
$p21^{Waf1/Cip1}$	Gastric epithelial cells	Up	EMSA; T-TF; ChIP	Chi et al. [2005]
KIR	NK cells	Down	EMSA	Trompeter et al. [2005]
CD36	Myeloid cells	Down	EMSA; T-TF; ChIP	Puig-Kröger et al., submitted [2005]
CCR7	Dendritic cells	Down	In vivo	Fainaru et al. [2005]
CD49d	Dendritic cells	Up	T-TF	Dominguez-Soto et al. [2005]
Syaliltransferase 1	Gastric cancer cells	Down	S-TF; Microarrays, N blot	Sakakura et al. [2005]
Chemokine receptor	Gastric cancer cells	Down	S-TF; Microarrays, N blot	Sakakura et al. [2005]
Galectin 4	Gastric cancer cells	Down	S-TF; Microarrays, N blot	Sakakura et al. [2005]
Desmoplakin	Gastric cancer cells	Down	S-TF; Microarrays, N blot	Sakakura et al. [2005]
CD26	Gastric cancer cells	Down	S-TF; Microarrays, N blot	Sakakura et al. [2005]
CD55	Gastric cancer cells	Down	S-TF; Microarrays, N blot	Sakakura et al. [2005]
Collagen type 13	Gastric cancer cells	Up	S-TF; Microarrays, N blot	Sakakura et al. [2005]
Collagen type 16	Gastric cancer cells	Up	S-TF; Microarrays, N blot	Sakakura et al. [2005]

The list includes genes whose regulatory regions have been shown to be regulated/bound by RUNX3, as well as genes whose expression changes upon alteration of RUNX3 levels either in vitro or in vivo.
ChIP, chromatin immunoprecipitation; EMSA, electrophoretic mobility shift assay; In vivo, transgenic/knockout mice; S-TF, stable transfection; T-TF, transient transfection; N Blot, Northern blot.

increases in metastatic tumors, what has allowed the establishment of a correlation between RUNX3 expression and survival [Sakakura et al., 2005; Wei et al., 2005]. Promoter hypermethylation is not the only mechanism responsible for the silencing of RUNX3 in cancer, as mutations that abolish the RUNX3 DNA-binding ability have been found in primary gastric tumor cells and bladder tumors [Li et al., 2002; Kim et al., 2005]. Recent data have also demonstrated RUNX3 subcellular mislocalization in 70% of RUNX3+ gastric cancers, where RUNX3 has an exclusive cytoplasmic localization and, consequently, a potentially impaired transcriptional activity [Ito et al., 2005].

RUNX3 maps to a chromosomal location (1p36) where loss of heterozygosity has been reported in some of these types of cancers. In agreement with this fact, and over the last 3 years, numerous reports have provided conclusive evidences that P2 promoter hypermethylation and reduced RUNX3 expression are not restricted to gastric cancer but can be detected in many tumor types including colorectal cancer, bile duct cancer, prostate cancer, lung cancer, pancreatic cancer, bladder cancer, and hepatocellular carcinoma (Table II). It is worth noting that silencing of RUNX3 expression correlates with P2 promoter CpG hypermethylation, whereas the pro-oncogenic potential of RUNX3 is related to retroviral insertions within the P1 promoter. In the absence of a detailed comparison of RUNX3 isoforms, it is currently unknown whether these promoter-specific effects reflect the existence of functional activities restricted to particular subsets of P1- or P2-derived RUNX3 isoforms.

TABLE II. RUNX3 Inactivation in Human Cancer

RUNX3 inactivation	Cancer type	Reference
P2 CpG hypermethylation	Bile duct	Wada et al. [2004]
	Bladder	Kim et al. [2005]
	Breast	Kim et al. [2004]
	Colorectal	Goel et al. [2004]; Ku et al. [2004]
	Endometrium	Kim et al. [2004]
	Esophagus	Schulmann et al. [2005]
	Gastric epithelium	Li et al. [2002]
	Hepatocellular	Mori et al. [2005]
	Larynx	Kim et al. [2004]
	Lung	Yanagawa et al. [2003]; Li et al. [2004b]
	Pancreas	Li et al. [2004a]
	Prostate	Kang et al. [2004]
	Testicular yolk sac	Kato et al. [2003]
	Uterin Cervix	Kang et al. [2005]
Point mutation	Bladder	Kim et al. [2005]
	Gastric epithelium	Li et al. [2002]
Protein mislocalization	Gastric epithelium	Ito et al. [2005]

Runx3−/− mice have been generated by two different laboratories. Both mouse models exhibit defects in neurogenesis, with altered development of dorsal root ganglion neurons [Inoue et al., 2002; Levanon et al., 2002], and defective T lymphocyte differentiation [Taniuchi et al., 2002; Woolf et al., 2003] (see below). However, the gastric epithelium hyperplasia seen in Runx3-deficient mice at birth by Li et al. [2002] has not been observed in an independent Runx3−/− mouse model [Fainaru et al., 2004], as these authors failed to detect any gastric defect at earlier time points during development [Brenner et al., 2004]. Instead, these second type of Runx3−/− mice develop inflammatory bowel disease with leukocyte infiltration and epithelial hyperplasia only after 4 weeks of age, and the hyperplasia of the glandular mucosa of the stomach appears at much later time points [Brenner et al., 2004]. These conflicting data can be due to the use of different background mouse strains, which might differ also in the expression of Runx3 in gastric epithelium, or to the use of different gene targeting constructs [Bae and Ito, 2003; Levanon et al., 2003]. In this regard, whereas the Runx3 KO mice generated by Ito's group might express a Runt–LacZ fusion protein that would function in a dominant negative manner [Bae and Ito, 2003; Levanon et al., 2003], mature dendritic cells and thymus from the Groner's Runx3−/− mice express a P1 promoter-derived exon2-lacking Runx3/p33 isoform [Fainaru et al., 2004], which is also upregulated during human dendritic cell maturation and which inhibits the activity of certain RUNX-dependent promoters in vitro [Puig-Kröger et al., unpublished results] (see below). Since RUNX3 is expressed by lymphoid and myeloid cells in the gastrointestinal tract, the possibility that the lack of RUNX3 expression in leukocytes might somehow contribute to the gastric lesions in Runx3−/− mice cannot be ruled out.

RUNX3 EXPRESSION IN IMMUNE CELLS

Since altered levels of RUNX proteins are associated with a variety of human diseases, determination of the mechanisms controlling

their expression, and activity is a matter of considerable interest. For RUNX3 expression, the importance of hypermethylation at the P2 promoter is further underscored by the fact that the inhibitor of methyltransferases 5-azaserine reactivates its expression in cancer cell lines [Sakakura et al., 2005]. Nuclease S1 protection assays on a panel of tissues have evidenced that the murine *Runx3* gene CpG-rich P2 proximal promoter is functional in most tissues, whereas distal promoter-derived transcripts are only detected in thymus and ovary [Rini and Calabi, 2001]. Consequently, RUNX3 can be found in numerous tissues and cell lineages, including cranial and dorsal root ganglia, cortex and medulla of the developing thymus, medulla of adult thymus, chondrocytes, and the mesenchyme of epidermal appendages [Levanon and Groner, 2004]. However, RUNX3 is predominantly expressed in cells of hematopoietic origin in adults, especially in myeloid, B- and T-cell lineages within the bone marrow, peripheral blood, spleen, and thymus [de Bruijn and Speck, 2004; Levanon and Groner, 2004], and can also be found in lymphoid B and myeloid cell lines [Le et al., 1999; Spender et al., 2002].

In B lymphocytes and certain lymphoid B-cell lines, TGF-β induces RUNX3 expression, whose constitutive levels correlate with the propensity of the B cells to switch to IgA β [Shi and Stavnezer, 1998], and EBNA-2 also induces RUNX3, whose presence correlates with an activated phenotype and is required for efficient proliferation of B cells immortalized by Epstein–Barr virus [Spender et al., 2002]. An interesting cross-regulation of RUNX1 and RUNX3 has been recently observed in human B lymphoid cell lines, where RUNX3 inhibits RUNX1 expression by repressing the RUNX1 P1 promoter, whereas siRNA-mediated down-regulation of RUNX3 enhances RUNX1 expression [Spender et al., 2005]. If this mechanism also operates in other cell lineages, the deregulated RUNX1 expression should be evaluated as a potential underlying cause for the pathological alterations observed in cells or tissues with defective or absent expression of RUNX3 (see below).

Within the lymphoid T lineage, RUNX3 is required for the correct development of CD8-lineage T lymphocytes. During T lymphocyte development, RUNX3 is initially detected in the double-positive ($CD4^+CD8^+$) thymocyte population and its expression increases during differentiation to the $CD4^-CD8^+$ single positive stage. Indeed, RUNX3 has been proposed as a master regulator for CD8 T lymphocyte lineage specification [Sato et al., 2005] because it potentiates CD8 and abrogates CD4 expression through binding to the CD8 gene enhancer and CD4 silencer regions, respectively. As a consequence, $CD4^-CD8^+$ thymocytes do not develop in the thymus of Runx3−/− mice because CD4 expression is de-repressed [Taniuchi et al., 2002; Woolf et al., 2003; Bosselut, 2004] and the number of $CD4^-CD8^+$ cells in Runx3-transgenic thymus is greatly increased [Kohu et al., 2005]. In addition, Runx3-deficient peripheral CD8 T cells express CD4 and exhibit defective responses to antigen [Taniuchi et al., 2002; Woolf et al., 2003], indicating that RUNX3 is involved in the maintenance of CD4 silencing in mature T cells, where it also activates transcription of the TCR-β gene [Meyers et al., 1996].

RUNX3 EXPRESSION IN MYELOID CELLS

Like RUNX1 and RUNX2 in other cell types, RUNX3 expression can be specifically and dose-dependently induced by three natural retinoids (ATRA, 13-*cis*-RA, and 9-*cis*-RA) in HL-60 myeloid cells. The failure of retinoic acid to induce RUNX3 in HL-60R cells, which harbor a dominant negative-like mutant of the RARα gene, indicates that RUNX3 expression is directly controlled by a retinoic acid-sensitive signaling pathway [Le et al., 1999], although the precise molecular mechanism remains to be determined. Whereas these compounds promote differentiation towards the granulocytic lineage [Le et al., 1999], it is currently unknown whether RUNX3 induction is a differentiation-dependent event. In this regard, RUNX3 protein expression is also enhanced by the differentiation-inducer PMA in both U937 and THP-1 myeloid cells, a phenomenon that can be confirmed by DNA-binding experiments [Puig-Kroger et al., 2003]. The RUNX3 upregulation in U937 cells correlates with an increase in CBFβ levels, indicating that protein stabilization, and not a transcriptional mechanism, might be responsible for this effect. This possibility is further supported by the finding that RUNX3 expression levels are dependent on three specific lysine residues within the C-terminal region of the Runt domain, and whose acetylation prevents ubiquitin-mediated RUNX3 degradation [Jin et al.,

2004]. Therefore, the expression of RUNX3 is regulated at a post-translational level, implying that the mere detection of mRNA for a particular RUNX3 isoform does not necessarily reflect the actual level of protein expression. In addition, this process might contribute to the increased RUNX3 expression in myeloid cell lines treated with differentiation-inducing agents [Le et al., 1999; Puig-Kroger et al., 2003].

So far, four RUNX3 isoforms have been described, all of which are detected in myeloid cells [Bangsow et al., 2001] (Fig. 1). The full-length RUNX3/p46 and p44 isoforms are derived from P1 and P2, respectively, whereas the alternatively spliced RUNX3/p33 and RUNX3/p27 isoforms are derived from promoter P1 and lack exon 2 (p33) or exons 2 and 5 (p27) [Bangsow et al., 2001; Fainaru et al., 2004] (Fig. 1). The RUNX3/p33 isoform, which lacks the exon encoding the N-terminal region of the Runt domain, exhibits a unique and restricted pattern of expression within adult human hematopoietic cells, although it can be detected in most lymphoid and myeloid leukemic cell lines [Puig-Kröger et al., unpublished results]. RUNX3/p33 expression is upregulated during maturation of murine bone marrow-derived dendritic cells [Fainaru et al., 2004] or human monocyte-derived dendritic cells [Puig-Kröger et al., unpublished results], while RUNX3/p27 exhibits a pattern of expression that partially overlaps that of RUNX3/p33 [Puig-Kröger et al., unpublished results]. It will be interesting to determine the transcriptional relevance of these isoforms in myeloid cells and specially that of RUNX3/p33, given the fact that a structurally similar isoform has been already described for RUNX1 (RUNX1δN) [Zhang et al., 1997].

RUNX3 FUNCTION IN MYELOID CELLS

Recently, the role of RUNX3 in myeloid dendritic cells has attracted much attention because of the functional alterations observed in murine Runx3−/− dendritic cells [Fainaru et al., 2004]. Dendritic cells are specialized in pathogen-sensing and antigen-presentation, with both effector functions displayed in a sequential manner and at distinct locations. Upon recognition of exogenous stimuli (pathogens, altered self, adjuvants), tissue-resident immature dendritic cells undergo profound alterations in their gene expression program (*Maturation*), which ultimately results in the loss of their pathogen-sensing ability and the acquisition of a strong ability to promote antigen-specific proliferation and polarization of naïve T lymphocytes. The available data on dendritic cell function indicate that T-cell interactions with immature dendritic cells lead to the induction of tolerogenic mechanisms, whereas T-cell interactions with pathogen-stimulated mature dendritic cells result in efficient activation of immune responses. On the other hand, the generation of pathogen-tailored immune responses is based on the flexibility of the dendritic cell maturation process, which includes "shared core response" and "pathogen-specific" alterations in the program of gene expression [Huang et al., 2001b]. Gene profiling experiments on human monocyte-derived dendritic cells have revealed that RUNX3 is one of the "core response" genes whose expression is transiently upregulated in response to a variety of pathogenic stimuli [Huang et al., 2001b]. Since RUNX3 is also greatly upregulated during maturation of murine bone marrow-derived dendritic cells [Fainaru et al., 2004], increased RUNX3 expression appears to constitute a hallmark of dendritic cell maturation. The NF-κB dependency of the dendritic cell maturation, the kinetics of RUNX3 upregulation during dendritic cell maturation, and the augmented IFN-dependent gene expression that takes place during this process points to RUNX3 as a direct target of NK-κB or IFN signaling pathways, a matter that deserves further investigation.

From a functional point of view, the altered effector functions of murine Runx3−/− dendritic cells clearly illustrate the relevance of RUNX3 in myeloid dendritic cell biology. Runx3−/− dendritic cells exhibit an accelerated maturation phenotype as well as increased T-cell stimulatory activity and altered β2 integrin expression. Since the agents that promote dendritic cell maturation (e.g., pathogens or adjuvants) are crucial for converting tolerogenic signals to activating signals, the altered maturation state of Runx3−/− dendritic cells strongly suggests that RUNX3 is a key factor in determining the final outcome of an immune response. In this regard, Runx3−/− develop spontaneous asthma-like symptoms and eosinophilic lung inflammation [Fainaru et al., 2004, 2005], and the loss of Runx3 function in leukocytes has been associated with

spontaneous inflammatory bowel disease with leukocyte infiltration, colitis, and gastric mucosal hyperplasia [Brenner et al., 2004]. As mentioned above, these immune disorders associated with Runx3 deficiency in dendritic cells could represent alternative, indirect explanation for the epithelial hyperplasia seen in Runx3-null mice. Considering all these facts, the identification of RUNX3 target genes in myeloid and epithelial cells (Table I) and the generation of conditional tissue-specific Runx3-deficient mice appear as feasible approaches to determine the precise relevance of RUNX3 downregulation in myeloid cells and other tissues.

The importance of RUNX3 for the proper acquisition of effector functions by myeloid dendritic cells can also explain the correlation established between certain single nucleotide polymorphisms and various autoimmune disorders. Three independent studies have identified genomic polymorphisms that predispose to systemic lupus erythematosus, rheumatoid arthritis, and psoriasis [Alarcon-Riquelme, 2003]. These polymorphisms are located within three genes (*SLC9A3R1*, *SLC22A4*, and *PDCD1*) encoded in different chomosomes but expressed in hematopoietic and immune tissues. More importantly, the three polymorphisms have the common molecular feature of affecting RUNX-binding DNA sequences, what has led to the proposal that RUNX factors have a central role in autoimmunity [Alarcon-Riquelme, 2003]. Given the critical involvement of dendritic cells for immune response regulation, and the elevated T-cell-stimulatory ability of Runx3−/− dendritic cells, it is reasonable to hypothesize that altered expression of RUNX3-dependent genes might impair the correct balance between tolerance and immunity and, therefore, predispose to immune diseases such as those mentioned above.

RUNX3 AND TGF-β SIGNALING IN MYELOID CELL DIFFERENTIATION

RUNX factors are not only regulated by members of the TGF-β family but are now considered as part of the TGF-β signaling pathways [Miyazono et al., 2004]. The connection between RUNX3 and TGF-β-initiated intracellular signaling has long been established in B lymphocytes. Besides its induction by TGF-β [Shi and Stavnezer, 1998], and the ability of TGF-β to induce nuclear translocation of RUNX3 [Ito et al., 2005], RUNX3 forms a complex with TGF-β-activated Smad3 and both factors stimulate transcription of the germline Ig Cα promoter in a cooperative manner [Zhang and Derynck, 2000]. The analysis of Runx3-null mice has provided conclusive evidences that the connection between RUNX3 and TGF-β is also operative in myeloid cells. Previous studies have demonstrated that generation of Langerhans cells (epidermal dendritic cells) is dependent on TGF-β [Borkowski et al., 1996], and that TGF-β exerts an inhibitory effect on the maturation of dendritic cells [Geissmann et al., 1999]. Like in the case of TGF-β−/− mice, the epidermis of Runx3−/− mice is completely devoid of Langerhans cells [Fainaru et al., 2004], and the lack of Runx3 prevents the inhibitory action of TGF-β on the LPS-induced maturation of bone marrow-derived dendritic cells [Fainaru et al., 2004]. Therefore, and although the expression of TGF-β and several components of the TGF-β signaling cascade (type I and II receptors, Smad2 and Smad3) is unaffected in Runx3−/− dendritic cells, these findings further emphasize the link between TGF-β signaling and RUNX3, and strongly suggest their joint contribution to the generation of this particular type of myeloid cells.

The relevance of the TGF-β/RUNX3 link within the myeloid lineage can be also inferred from studies performed on in vitro generated dendritic cells. First, the presence of TGF-β during the generation of monocyte-derived dendritic cells results in the generation of cells with phenotypic properties of Langerhans cells including the expression of E-Cadherin [Geissmann et al., 1998], which has been identified as a RUNX3 target in gene profiling experiments in distinct cell lineages. Second, RUNX3 regulates the expression of the integrin αE (CD103) [Grueter et al., 2005], which is also specifically expressed on a subset of dendritic cells in peripheral lymph nodes. Third, TGF-β has long been known to alter the expression of integrin molecules involved in dendritic cell migration (α5, α4, αL, αX) [Ignotz et al., 1989], and whose gene regulatory regions are responsive to RUNX3 overexpression [Puig-Kroger et al., 2003; Dominguez-Soto et al., 2005]. Finally, the finding that the Notch ligand Delta-1 signaling cooperates with TGF-β in inducing the differentiation of human monocytes into E-Cadherin + Langerhans cells [Hoshino et al.,

2005] is in line with the physical and functional cooperation of Notch downstream targets and RUNX factors and the recent suggestions that RUNX factors can modulate the Notch signaling pathway [Tsai and Gergen, 1995; Burns et al., 2005]. Given all these considerations, the determination of RUNX3 target genes will be a valuable approach to identify the molecules directly implicated in Langerhans cells specification and to define their functional maturation state.

The contribution of RUNX3 to the development and maturation of myeloid dendritic cells has other potential implications. On the one hand, since the transcription factors C/EBPα and PU.1 physically interact with the Runt domain of RUNX1 [Ito, 2004] and have been demonstrated to promote and inhibit, respectively, the generation of Langerhans cells from human myeloid progenitors [Iwama et al., 2002], it is possible that the incidence of both factors on Langerhans cells development is mediated via interactions with RUNX3. On the other hand, the involvement of RUNX3 in TGF-β signaling in myeloid cells also suggests its participation in the alternative pathway of macrophage activation. Classically activated macrophages (CAMØ) eradicate invading microorganisms and tumor cells, and promote Th1 responses, while the resolution of inflammation is carried out by alternatively activated macrophages (AAMØ), which are mainly involved in debris scavenging, tissue remodeling, extracellular matrix production, and wound healing [Mantovani et al., 2002]. Thus, AAMØ exhibit activities, which are known to be mediated by TGF-β in numerous cell types. Besides, the presence of TGF-β is required for in vitro differentiation of AAMØ [Rauh et al., 2005]. Therefore, RUNX3 might be partially responsible for the phenotype and effector functions acquired along the distinct pathways of macrophage activation (classic vs. alternative), a hypothesis further suggested by the differential expression of CD36, a RUNX3-regulated gene, in both types of macrophages [Puig-Kröger et al., submitted for publication, 2005] (Fig. 2).

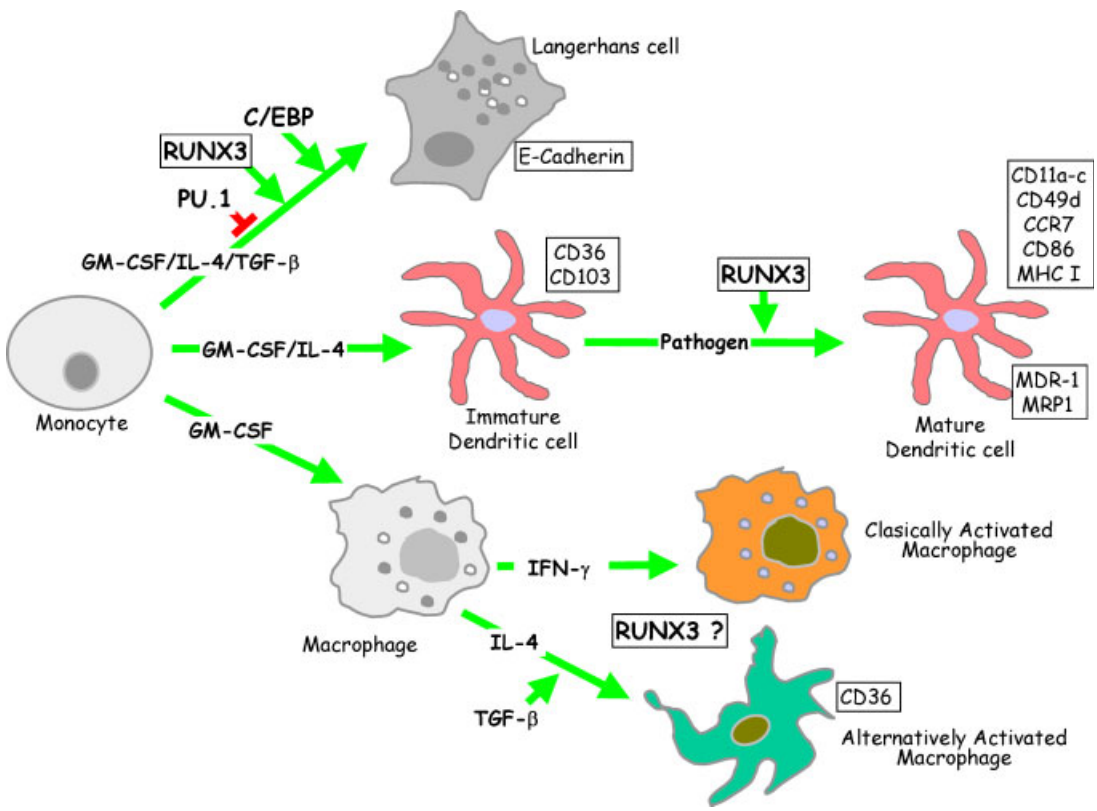

Fig. 2. Hypothetical contribution of RUNX3 to the major monocyte in vitro differentiation pathways. RUNX3-dependent genes whose expression marks discrete differentiation stages are indicated. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CELL SIGNALING PATHWAYS AFFECTING RUNX3 FUNCTIONAL ACTIVITY

Apart from its multilevel cooperation with the TGF-β signaling pathway [Miyazono et al., 2004], little information is available about the extracellular stimuli and signaling pathways controlling RUNX3 functions (DNA-binding or transcriptional activity), probably because its physiological relevance has been only recently put forward. However, some predictions could be inferred from studies done on both RUNX1 or RUNX2. ERK-dependent phosphorylation enhances RUNX1 transactivation by preventing its association with the mSin3A corepressor [Imai et al., 2004], whereas ERK and PI3K signaling pathways modulate the activity RUNX2, whose phosphorylation at two particular serine residues determines both heterodimerization and functional mobilization [Fujita et al., 2004; Qiao et al., 2004]. By contrast, RUNX3 lacks the region containing the serine residues phosphorylated by ERK in RUNX1 and RUNX2, suggesting that modulation of RUNX3 activity by intracellular signaling has some unique features. As mentioned above, a new level of regulation of RUNX3 has been recently revealed by showing that its ubiquitin-mediated degradation is prevented by TGFβ-induced acetylation, a process which involves the transcriptional co-activator p300 [Jin et al., 2004]. Predictably, the analysis of RUNX-based reporter constructs in cells expressing individual members of the RUNX family may constitute a valid approach to evaluate the signaling pathways affecting RUNX3 transcriptional activity.

Since RUNX proteins are implicated in numerous physical and functional interactions, it is reasonable to postulate that their transcriptional activity could be also modulated by signaling pathways affecting the stability or the activity of those RUNX-interacting factors. As an example, the analysis of the contribution of RUNX3 to the CD11a integrin expression has revealed that PMA-induced differentiation correlates with a switch in the occupancy of a critical DNA element within the CD11a integrin promoter, where RUNX3 competitively replaces C/EBPα only at the later stages of differentiation [Puig-Kroger et al., 2003]. Therefore, it can be hypothesized that the RUNX3-dependent transcription would be affected by intracellular signals directly acting on the level of expression or the activity of C/EBP family members. Such a conclusions could be also drawn from the cooperative interactions of RUNX and C/EBP family members that take place on the regulatory regions of numerous myeloid genes [Ito, 2004].

RUNX3 AND CELL ADHESION AND MIGRATION

A link between RUNX activity and cell adhesion has become evident from studies on distinct cellular differentiation systems. RUNX2 regulates migration and invasion in vascular endothelial cells, and forced expression of a dominant negative RUNX2 construct inhibits cell migration of endothelial cells [Fujita et al., 2004] and osteoblastic cell lines [Sun et al., 2001]. The participation of RUNX3 in the regulated expression of adhesion molecules has now been noted in several cellular lineages. In the myeloid lineage, RUNX3 regulates the expression of adhesion molecules and chemokine receptors involved in cell migration (αL, αX, α4 integrins, CCR7, CD36) [Dominguez-Soto et al., 2005; Fainaru et al., 2005], and in the formation of the immunological synapse (αL integrin) [Puig-Kroger et al., 2003; Fainaru et al., 2004]. Moreover, comparison of the transcriptome in myeloid U937 cells overexpressing either prototypic RUNX3 or the RUNX3p33 isoform has revealed that a significant number of differentially expressed genes are involved in cell–cell adhesion and cell migration [Puig-Kröger et al., unpublished results]. In the lymphoid lineage, murine Runx3, in collaboration with TGF-β, is necessary to induce expression of the E-Cadherin-interacting integrin αE during $CD8^+$ thymocyte development [Grueter et al., 2005]. Regarding other cell lineages, overexpression of RUNX3 in the KATO-III gastric cancer cell line results in altered expression of genes involved in cell adhesion and migration [Sakakura et al., 2005], and a similar approach using the SGC7901 gastric cancer cell line has revealed that the two multidrug resistance family members MDR-1 and MRP1 are downregulated by RUNX3 [Guo et al., 2005]. While this finding suggests that RUNX3 could modulate cellular sensitivity to chemotherapy, it has an additional implication for myeloid cell adhesion and migration, since both MDR-1 and MRP1 participate in dendritic cell migration from skin via

afferent lymphatics [Randolph et al., 1998] and in augmentation of the chemotactic responsiveness to CCL19 in dendritic cells [Robbiani et al., 2000]. Altogether, these set of data strongly suggest that RUNX3 is an important contributor to the profile of adhesion molecules expressed by hematopoietic, and specially myeloid lineage cells.

Conversely, adhesion also appears to modulate the activity of RUNX factors, although this effect has only been demonstrated in the case of RUNX2. Integrin α2-collagen interactions can enhance RUNX2 DNA-binding activity as a previous step for osteoblast-specific gene expression [Xiao et al., 1998], and RUNX2 mediates the osteogenic gene expression program triggered upon laminin 5 binding to integrin α3/β1 [Klees et al., 2005]. Thus, apart from the effects of RUNX3 on the expression of adhesion-related genes, this set of data raises the interesting possibility that adhesion itself might also regulate the activity of RUNX3, which is preferentially expressed in cells (hematopoietic lineage) whose adhesive interactions are dynamically regulated and determine the final outcome of immune and inflammatory responses.

CONCLUDING REMARKS

RUNX3 is the least-studied member of the RUNX family of transcriptional regulators. Recent reports, however, have attracted much attention to this factor because of the pleiotropic effects caused by its deficiency in mice, and which point to RUNX3 as a tumor-suppressor gene as well as a relevant player in myeloid cell gene expression. Regarding this latter issue, fine-tuned RUNX3 expression appears to be critical in dendritic cell biology, and its upregulated expression can even be considered as a hallmark for dendritic cell functional maturation. The essential involvement of dendritic cells in immune tolerance and immune response, inflammation and tumor immunosurveillance, and the altered effector functions observed in murine Runx3−/− dendritic cells, makes the identification of RUNX3 target genes as one of the main goals in the field. From this point of view, gene profiling analysis, chromatin immunoprecipitation experiments (ChIP-on-chip) and the use of RUNX3 siRNA appear as reasonable approaches to identify the genes and the functions specifically controlled by RUNX3 in myeloid dendritic cells, as well as to clarify whether the various alterations observed in Runx3−/− mice have a common molecular basis or reflect tissue/cell-specific activities of RUNX3. Besides, the generation of conditional and tissue-specific will be very helpful to determine the extent of implication of myeloid cell-autonomous Runx3 function in the alterations currently seen in Runx3 deficient tissues and in the genesis of autoimmune disorders. In any event, these and other future approaches involving the modulation (overexpression, knockdown or abrogation) of RUNX3 expression should take into account the existence of alternatively spliced isoforms of RUNX3, whose activity, pattern of expression and tissue-distribution must be accurately determined before any conclusion can be drawn.

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