Oncogene (2014) **33,** 403–410

www.nature.com/onc

REVIEW

Myocyte enhancer factor 2C in hematopoiesis and leukemia

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MEF2C is a selectively expressed transcription factor involved in different transcriptional complexes. Originally identified as an essential regulator of muscle development, ectopic expression of MEF2C as a result of chromosomal rearrangements is now linked to leukemia. Specifically, high MEF2C expression has been linked to mixed lineage leukemia-rearranged acute myeloid leukemia as well as to the immature subgroup of T-cell acute lymphoblastic leukemia. This review focuses on the role of MEF2C in the hematopoietic system and on aberrant MEF2C expression in human leukemia.

Oncogene (2014) **33,** 403–410; doi:10.1038/onc.2013.56; published online 25 February 2013

Keywords: MEF2C; hematopoiesis; leukemia

THE MYOCYTE ENHANCER FACTOR 2 (MEF2) FAMILY AND ITS CONSERVED DOMAINS

The vertebrate MEF2 (also referred to as myocyte enhancing factor 2) group of proteins belong to the MADS box (MCM1-agamous-deficiens-serum response factor) family of transcription factors and consists of four family members MEF2A, B, C and D (reviewed in Black and Olson[1]). The MEF2 family members have multiple splice variants and share a conserved N-terminal MADS box and a MEF domain (Figure 1). These domains are required for DNA binding in promoter regions of muscle-specific genes and for dimerization and interaction with myogenic basic helix–loop–helix (bHLH) proteins.[2,3] Thus, MEF2 transcription factors that form a complex with myogenic bHLH proteins regulate muscle differentiation.[4,5] The highest expression of *Mef2c* is found in skeletal muscle, heart, brain and spleen.[6–8] *Mef2c* variants contain α1 or α2 exons in a mutual exclusive manner. The *α2-Mef2c* variant is primarily expressed in striated (skeletal) muscle, whereas the α1 isoform is expressed in other tissues.[9] The *Mef2c* isoform including the β exon in the second transactivation domain has enhanced transactivation potential, and is expressed in neuronal tissues including the brain.[8,9] The γ region present in some MEF2C isoforms functions as a repressor of the transcriptional activity of MEF2C and is predominantly spliced out in many tissues due to the unique presence of a 3′-splice acceptor site in *MEF2C*, but not other *MEF2* genes (Figure 1). The repressive activity of the γ domain is conferred by phosphorylation of serine 396 (S396), and is abolished when this serine is replaced by another amino acid.[6] Phosphorylation at S396 facilitates sumoylation at lysine 391 (K391) of MEF2C, which facilitates recruitment of unknown co-repressors to inhibit transcription.[10]

MEF2C IS ESSENTIAL FOR THE DEVELOPMENT OF MANY CELL TYPES

At the onset of cardiac and skeletal muscle lineage differentiation during mouse embryogenesis, *Mef2c* is expressed first, followed by the other Mef2 genes.[11] In the absence of Mef2c, mice die at embryonic day E9.5 due to cardiovascular defects.[12] A *LacZ* reporter transgenic mouse model driven by the *Mef2c* control region revealed that *Mef2c* is a direct transcriptional target of myogenic bHLH and MEF2 proteins during skeletal muscle development. The bHLH DNA-binding site is necessary for initiation of *Mef2c* expression, whereas an adjacent MEF2-binding site is required for the maintenance of *Mef2c* expression.[13] Besides its role in cardiac and skeletal muscle differentiation, conditional knockout of Mef2c in various tissues has revealed a role for Mef2c in bone development[14] and osteoclast-mediated bone resorption.[15] In addition, *in vivo* models have established roles for Mef2c in neuronal development.[16–20] In line with this, some neurobehavioral phenotypes, including mental retardation, epilepsy and autism spectrum disorders in humans, have been linked to *MEF2C* haploinsufficiency due to mutations in or deletions of *MEF2C*.[21–24] Finally, Mef2c is required for craniofacial and melanocyte development.[25,26]

MEF2C PARTICIPATES IN DIFFERENT TRANSCRIPTION FACTOR COMPLEXES

Biochemical analysis in differentiating myocyte and fibroblast cell lines revealed that overexpressed bHLH myogenin and MEF2C cooperate in the direct E-box-mediated transcriptional activation of the *MLP* gene (muscle LIM-only (LMO) protein).[27] MLP promotes myogenesis by enhancing the activity of the muscle bHLH factor MyoD through binding of MLP to MyoD.[28] Thus, in muscle development, the muscle-specific MyoD and myogenin determine muscle cell fate and differentiation, whereas the non-muscle-specific MEF2C serves as an essential cofactor that binds E2A-bound MyoD or myogenin to enhance E-box-dependent muscle-specific gene transcription[4] (Figure 2a). MEF2C and myogenic factors can each bind DNA and activate transcription via their activation domains. Through its activation domain, MyoD also binds and recruits the coactivator histone acetyltransferase p300, thus enhancing E-box-dependent transcription.[29] In addition to the direct interaction with myogenic factors, the MADS box of MEF2C can bind and recruit p300 as well.[29] Acetylation of MEF2C by p300 enhances DNA-binding activity and myogenic differentiation.[30] Another potent coactivator is the steroid nuclear receptor coactivator of transcription NCOA2/GRIP-1 of which the

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Received 10 December 2012; revised 17 January 2013; accepted 18 January 2013; published online 25 February 2013

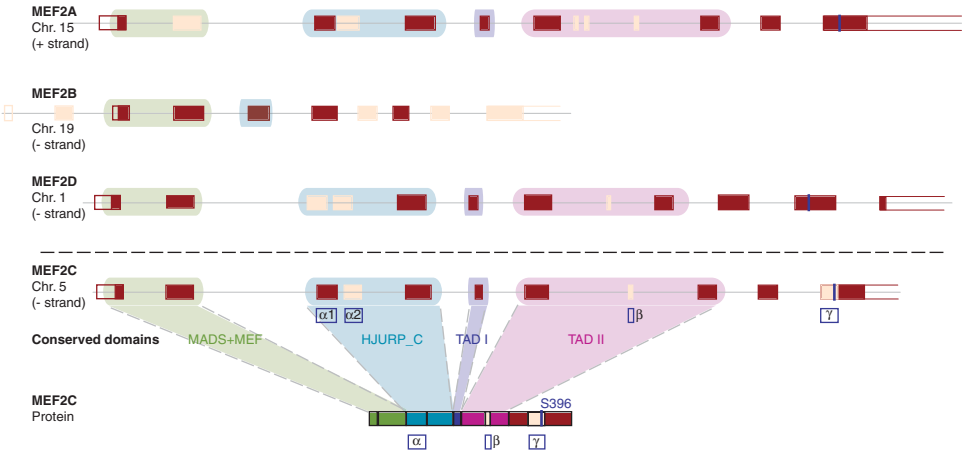

Figure 1. Schematic representation of the four homologous genes *MEF2A–D*. *MEF2A*, *B* and *D* genes are compared with *MEF2C* (below the dashed line) with 5′-UTR (left) and 3′-UTR (right) indicated as open boxes. Solid dark red boxes: exons; light beige boxes: alternatively spliced exons. Conserved domains of the *MEF2A*, *B*, *C* and *D* genes are indicated as opaque regions corresponding with the solid colors of the MEF2C protein domains (bottom). Green: MADS and MEF domains; light blue: HJURP_C (Holliday junction regulator protein family C-terminal repeat); dark blue: transactivation domain I; pink: transactivation domain II.

N-terminal bHLH-PAS domain directly binds the bHLH region of myogenic factors, whereas the C-terminal activation domain binds the MADS domain of MEF2C.[31] *In vitro* transcription and translation followed by immunoprecipitation assays imply that myogenin, E2A and MEF2C physically interact in a complex with the coactivators NCOA2/GRIP-1 and p300 during myogenesis[31] (Figure 2b).

Another interaction with MEF2 proteins involves class II histone deacytelases HDAC4 and HDAC5 together with other co-repressors. HDAC4 and HDAC5 both contain an N-terminal MEF2-binding site, which is lacking in HDAC1 or HDAC3. This HDAC4/5-MEF2C interaction provides a mechanism to repress MEF2C-regulated transcription that can be relieved through nuclear export of HDACs. Release from MEF2C and subsequent nuclear export relies on the phosphorylation of HDAC4 and HDAC5 by the Ca^{2+}/Calmodulin-dependent protein kinase[32,33] (Figure 2b).

In T cells, Cabin1 is an inhibitor of apoptosis, and acts by directly binding to the MADS-MEF domain of MEF2 resulting in the repression of the nuclear steroid receptor *Nur77*. Overexpression of Cabin1 and MEF2 in Jurkat T cells revealed the interaction of Cabin1 with MEF2B[34] and with MEF2D,[35] but Cabin1 can actually bind all four MEF2 proteins.[34] The mechanism of Cabin1-mediated transcriptional repression is twofold: Cabin1 recruits both the repressor mSin3 and associated class I HDAC1 and HDAC2 to MEF2 and competes with the coactivator p300 acetyltransferase for binding the N-terminal MADS-MEF domain of MEF2 (Figure 2c). Ca^{2+} signaling results in a release of Cabin1 from MEF2 and in the activation of MEF2-dependent transcription, due to competitive binding of Ca^{2+}/Calmodulin to Cabin1.[34] Binding of Ca^{2+}/Calmodulin to Cabin1 results in the removal of the inhibitory HDAC complex from MEF2, allowing p300 to associate with MEF2 to drive transcription.[35] Unlike the class IIa HDAC4, -5, -7 and -9, which bind the MEF2 domain, class I HDAC3 interacts with the MADS box domain and can deacytelate MEF2, providing an additional layer of repression of MEF2-dependent transcription.[36–38] PC4/IFRD1/Tis7 is another coactivator of MyoD that promotes MEF2C-dependent transcription by binding and removing HDAC4[39] and HDAC3[40] from the complex (Figure 2b).

Mastermind-like transcriptional coactivator (MAML) coactivates the NOTCH signaling pathway by binding cleaved intracellular NOTCH (ICN), which together with the transcription factor CSL (CBF1, Suppressor of Hairless, Lag-1) form the core components of the active NOTCH transcriptional complex. MAML1 is involved in active transcriptional complexes other than ICN–CSL, one of which is the MEF2C-dependent transcription complex (Figure 2d). The first indication for the MAML1–MEF2C interaction came with the finding that the *Maml1* knockout mice have a severe muscular dystrophy-like phenotype with perturbation of overall skeletal muscle structure.[41] MAML1 is essential for muscle gene expression and in this context binds and coactivates MEF2C together with p300. However, active NOTCH signaling represses myogenesis,[42] and Notch3 and Mef2c function antagonistically in differentiating myoblasts,[43] providing a mechanism to inhibit MEF2C-mediated transcription via competitive recruitment of MAML1 to ICN upon NOTCH activation.[44,45] It is unknown whether the functional switch of MAML1 is regulated through competitive binding between ICN and MEF2C, or that MAML1 interacts with both proteins simultaneously. Also, it is unknown whether the MAML1–MEF2C interaction is direct or indirect.[44] Besides competitive binding of MAML1 by ICN, ICN can also inhibit muscle development by direct interaction with a region adjacent to the MEF DNA-binding domain uniquely present in the α1 domain of MEF2C (which is lacking in MEF2A, MEF2B and MEF2D proteins), resulting in a block of MEF2C DNA- and MyoD/Myogenin-binding[46] (Figure 2e). In *Drosophila*, ICN and MEF2C act synergistically on activation of Jun N-terminal kinase and matrix metalloproteinase 1 expression levels, thereby promoting proliferation and metastasis.[47] It is unknown whether activated NOTCH and Mef2 need to interact directly, or depend on the recruitment of Maml or other molecules. High levels of MEF2 and NOTCH correlate in human metastatic breast cancer, so a potential functional synergistic action of these proteins in human tumorigenesis needs further investigation.[47]

EXPRESSION AND FUNCTION OF MEF2C IN THE HEMATOPOIETIC COMPARTMENT

Mef2c is differentially expressed in various stages of hematopoietic development, as summarized in Figure 3a. In both hematopoietic stem cells (HSCs) and common myeloid progenitors (CMPs), *Mef2c* is abundantly expressed.[48,49] *Mef2c* expression decreases during myeloid maturation into more committed cells, including granulocyte–monocyte progenitors (GMPs) and megakaryocyte–erythroid progenitors. In common lymphoid precursors (CLPs), *Mef2c* expression is highly abundant as compared with HSC and CMP and decreases somewhat when cells commit to the B-cell lineage. In contrast, *Mef2c* expression is

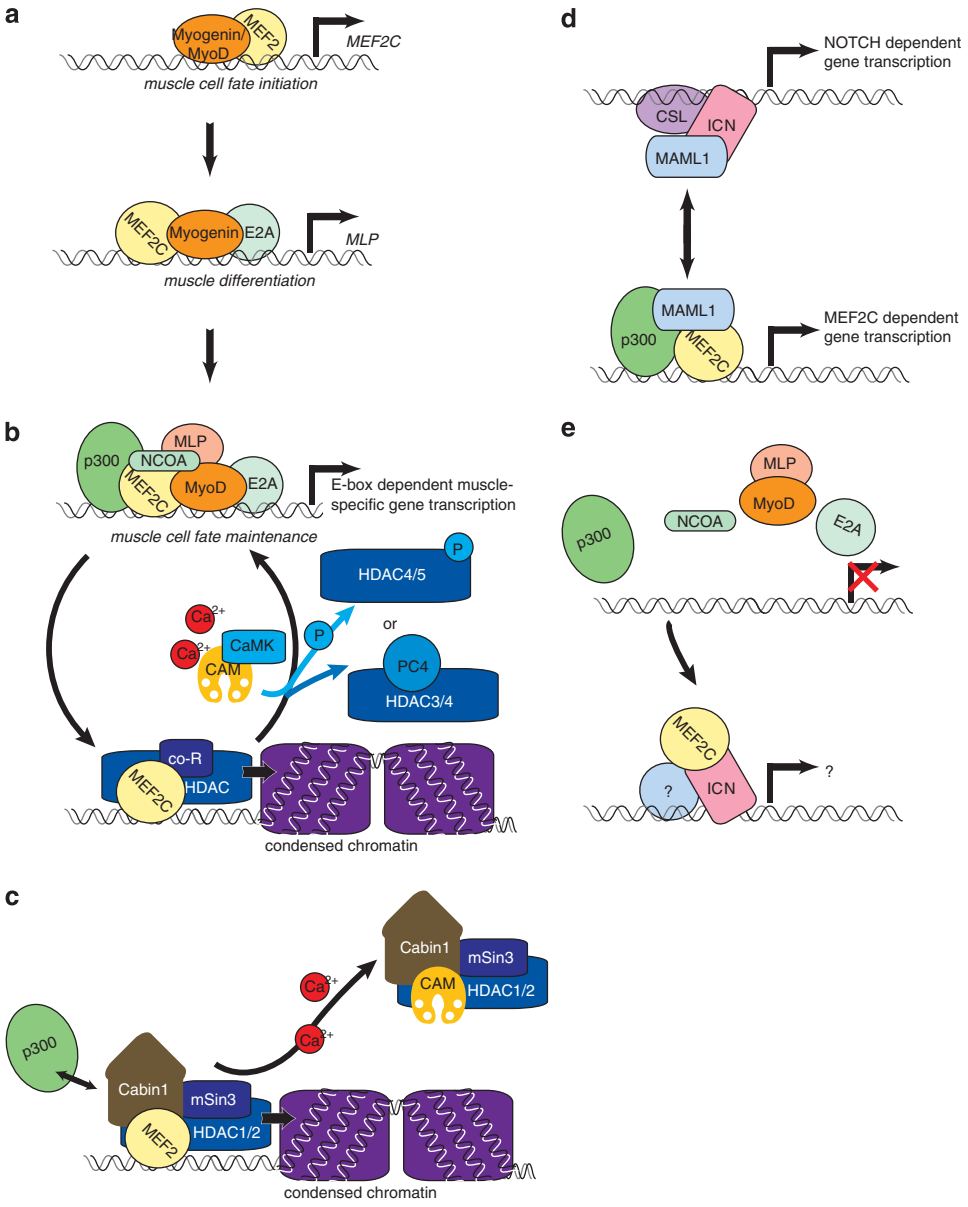

Figure 2. MEF2C functions in different transcription factor complexes. (**a**) MEF2C is a key component in muscle-specific gene transcription. (**b**) MEF2C recruits coactivators p300 and NCOA to enhance and stabilize transcription. HDAC and other co-repressors compete for MEF2C interaction and repress transcription. (**c**) In T cells, Cabin1 functions as a co-repressor of MEF2-dependent transcription. (**d**) Competitive binding of MAML1 between MEF2C and NOTCH transcription complexes. (**e**) Activated NOTCH (ICN) interaction with MEF2C blocks MEF2C DNA-binding ability and MEF2C-dependent transcription.

virtually absent in T cells.[48–50] Differential expression may be in line with differential requirement for Mef2c at various stages of hematopoiesis, as is best characterized in a conditional knockout mouse model.[51]

Several groups have used mice in which exon 2 of *Mef2c* is flanked by loxP sites, crossed with Cre recombinase transgenic mice to delete Mef2c at various stages of hematopoietic development. A role for Mef2c in monocyte differentiation was demonstrated using $Mef2c^{f/-} \times Mx\text{-}Cre^{+}$ mice, in which *Mef2c* was deleted upon polyI:C-induced *Mx-Cre* transgene expression.[48] Mef2c is not required for the establishment or maintenance of the myeloid lineage *in vivo*, as no cell number differences in CMP, GMP and megakaryocyte–erythroid progenitor compartments were observed using this mouse model. *In vivo*, only a slight reduction of the number of monocytes was found in the absence of Mef2c. In line with this, *in vitro* colony-forming unit assays revealed a twofold reduction in the number of monocyte colonies from Mef2c-deficient bone marrow as compared with wild-type bone marrow. Conversely, constitutive overexpression of Mef2c in immature, lineage-negative bone marrow cells resulted in a severe reduction of $Gr1^{+}$ granulocyte numbers (and higher monocyte percentages) in methylcellulose culture systems in the presence of either macrophage colony-stimulating factor or granulocyte colony-stimulating factor, which was attributed to the Mef2c target *c-Jun*.[48] Similarly, ectopic expression of constitutively active Mef2c in CMPs resulted in a severe reduction of $Gr1^{+}$ $Mac1^{+}$ mature myeloid cell numbers in OP9-stroma support culture systems with myeloid-promoting cytokines.[49] Because the effects of Mef2c deficiency on the myeloid compartment *in vivo* are minimal, the authors suggest that the Mef2c function to induce monocyte differentiation is most evident under cytokine-induced (for example, stress) circumstances and that Mef2c is important in

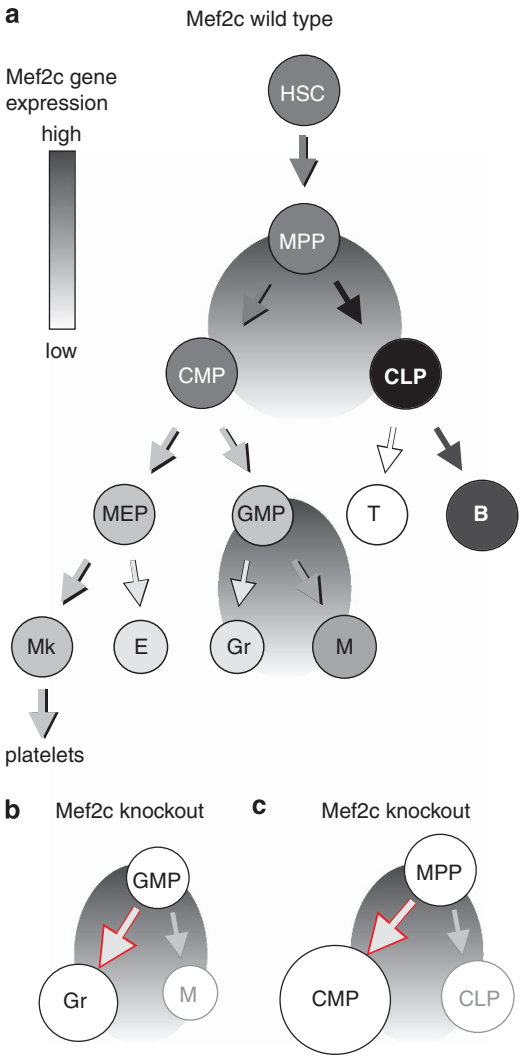

Figure 3. Mef2c gene expression in the different hematopoietic lineages and the effect on lineage choice. (**a**) Relative Mef2c gene expression is indicated by using a gray scale (white: no expression, black: highest expression in hematopoietic cells).[48,49] B, B lymphocyte; CMP: common myeloid progenitor; CLP: common lymphoid progenitor; E, erythrocyte; Gr, granulocyte; GMP, granulocyte–monocyte/macrophage progenitor; HSC, hematopoietic stem cell; M, monocyte; MEP: megakaryocyte–erythrocyte progenitor; MPP: multipotent progenitor; Mk: megakaryocyte; T, T lymphocyte. (**b**) Skewing from monocyte to granulocyte development in the absence of Mef2c. (**c**) Skewing from CLP to CMP in the absence of Mef2c.

modulating the myeloid cell fate decision between monocyte and granulocyte differentiation[48] (Figures 3a and b).

Granulocyte differentiation is regulated by microRNA-223. MiR-223 expression is regulated by the transcription factors NFI-A and C/EBPα that compete for miR-223 promoter binding.[52] C/EBPα induces miR-223 expression, whereas NFI-A inhibits miR-223 expression levels. Moreover, *Mef2c* is one of the directly repressed targets of miR-223.[53] Remarkably, miR-223 knockout (*miR-223*$^{-/Y}$) mice have increased numbers of circulating neutrophils and an expanded GMP compartment. To show that this phenotype was a result of increased Mef2c levels, *miR-223*$^{-/Y}$ × *Mef2c*$^{f/f}$ double knockout mice were used, in which *Mef2c* is deleted by the myeloid-specific lysozyme M-Cre. As expected, the GMP expansion and increased numbers of neutrophils are not observed in the double knockout mice. Therefore, elevated expression of Mef2c levels in miR-223 knockout mice has a positive effect on granulopoiesis,[53] in contrast to the *Mef2c*$^{f/-}$ × *Mx-Cre*$^{+}$ mouse model.[48] In this respect, it cannot be excluded that miR-223 regulates various other targets besides *Mef2c* that may impact on granulopoiesis.

Mef2c$^{f/f}$ × *Vav-Cre*$^{+}$ mice, in which Mef2c expression is knocked out from all hematopoietic lineages including HSCs, have greatly affected megakaryocyte development as measured by defects in platelet size, shape, granularity and total counts. In addition, 6- to 12-month-old mice have a slight increase in neutrophil numbers and a slight decrease in total lymphocyte numbers in the absence of Mef2c.[54]

Vav-Cre-mediated deletion of *Mef2c* does not change the numbers of circulating T cells, but results in mild defects in pre-B-cell development in the bone marrow and reduced numbers of peripheral blood B cells.[54] Largely consistent with this model, *Mef2c*$^{f/f}$ × *CD19-Cre*$^{+}$ mice to specifically knockout Mef2c in B cells reveal that Mef2c is involved in B-cell homeostasis/survival, germinal center formation in the spleen and in proliferation upon B-cell receptor (BCR) stimulation.[55,56] Both studies conclude that an efficient B-cell activation upon antigen-dependent BCR stimulation (adaptive immune response) requires Mef2c, and that non-BCR-mediated pathways of B-cell proliferation (innate immune response) are independent of Mef2c. However, one report places BCR-induced Mef2c signaling downstream of p38MAPK, which phosphorylates and activates Mef2c,[55] whereas the other suggests Mef2c is activated by the Ca^{2+}-dependent Calcineurin-Calmodulin pathway.[56] Both Mef2c-deficient B cells[56] and B cells lacking Calcineurin activity[57] have defects in proliferation following BCR stimulation correlating with a lack of Cyclin D2 induction. Although the number of peripheral blood B cells is lower in *Mef2c*$^{f/f}$ × *Vav-Cre*$^{+}$ mice, the percentages and localization of B-cell subsets are normal in *Mef2c*$^{f/f}$ × *CD19-Cre*$^{+}$ mice, suggesting that B-cell homing and differentiation is independent of Mef2c. Early pre-B-cell percentages in the bone marrow are also comparable between *Mef2c*$^{f/f}$ × *CD19-Cre*$^{+}$ and control mice,[56] in contrast to the *Mef2c*$^{f/f}$ × *Vav-Cre*$^{+}$ mouse model[54] and a model in which Mef2c is deleted during the early stages of immunoglobulin rearrangements (by Mb-1-Cre).[58] This discrepancy is explained by the fact that CD19-Cre is not fully active during early B-cell development in the bone marrow.

In addition to modulating monocyte-versus-granulocyte differentiation in the myeloid lineage,[48] Mef2c also regulates the decision point between the lymphoid and myeloid lineage[49] (Figures 3a and c). Multipotent progenitors (MPPs) from *Mef2c*$^{f/f}$ × *Mx-Cre*$^{+}$ mice promote myeloid development when cultured on OP9-stroma with cytokines that would normally promote lymphoid development: control MPPs only give rise to $CD19^{+}$ pro-B cells, whereas Mef2c-deficient MPPs also yield a significant percentage of Gr^{+} granulocytes.[49] In the Mef2c-deficient bone marrow of these *Mef2c*$^{f/f}$ × *Mx-Cre*$^{+}$ mice, the percentage of CLPs is significantly reduced, whereas percentages of HSCs and lymphoid-primed MPPs are not affected in the absence of Mef2c. Microarray analysis of *Mef2c*-deleted MPPs revealed down-regulation of many genes that are involved in lymphopoiesis (such as *Tcf7*, *Ets1*, *Gata3*, *Rag1*). Remarkably, the myeloid transcription factor *Cebpa* (among others) was upregulated in the absence of Mef2c, possibly indicating an antagonizing mechanism between Mef2c (which promotes lymphopoiesis) and C/EBPα (which promotes myelopoiesis). Moreover, *Mef2c* is a downstream target of the hematopoietic transcription factor PU.1.[49] Although the direct effect of *Mef2c* deletion from the hematopoietic compartment using Mx-Cre is small and difficult to measure, an impressive impairment of lymphoid development is seen in a competitive chimeric transplantation setting. Co-transplantation of Mef2c-deficient and control HSCs (lineage-negative bone marrow) results in peripheral hematopoietic engraftment with a severe skewing of Mef2c-deficient cells

toward monocytes and granulocytes at the expense of B-, T- and NK cells.[49]

Taken together, these mouse models have revealed roles for Mef2c in regulating the precursor cell commitment toward lymphoid development over myeloid development and toward monocyte differentiation over granulocyte development within the myeloid lineage. Furthermore, Mef2c is important in the development of megakaryocytes and B cells (Figure 3).

MEF2C IN ACUTE MYELOID LEUKEMIA (AML)

Acute leukemia is a cancer of developing hematopoietic cells. Genetic aberrations ranging from chromosomal translocations to point mutations can lead to the oncogenic transformation of these cells. For myeloid leukemia, initiation and maintenance requires the ability of at least a subpopulation to self-renew, and this population of leukemic cells shares self-renewal capacity with normal HSCs. Hence, this self-renewing leukemic population is referred to as leukemic stem cells (LSCs). In a mixed lineage leukemia (*MLL*-rearranged) AML mouse model, committed GMPs were transduced with the *MLL–AF9* fusion oncogene and transplanted into recipient mice to induce leukemia.[59] This model illustrates that committed GMPs can become LSCs by acquiring a self-renewal-associated gene expression signature while maintaining the committed progenitor signature. Gene expression comparison of isolated leukemic *MLL-AF9* GMPs versus control GMPs reveals a self-renewal signature consisting of genes normally involved in HSC development (including several homeobox genes) and also includes induction of *Mef2c*. Leukemic *MLL-AF9* GMPs contain a high frequency of leukemia-initiating cells as shown by secondary transplantations and *in vitro* colony formation assays. Leukemic *MLL-AF9* GMP colony formation was reduced by 90% upon knockdown of *Mef2c* expression. Secondary injection of leukemic GMP colonies into recipient mice resulted in an AML-induced mortality rate of 56%. However, only 20% mice died of AML when transplanted with leukemic GMP cells in which *Mef2c* expression had been knocked down.[59] This indicates that *Mef2c* is involved in LSC development in *MLL*-rearranged AML. As *Mef2c* is highly expressed in HSC and in early CMP and CLP progenitors and as its expression correlates with (a differentiation arrest at the stage of) an immature phenotype that is directly activated by MLL-AF9, Mef2c may be important in conferring HSC features to LSCs.[59] Although Mef2c has a role in leukemogenesis[60,61] by providing a differentiation arrest, it is insufficient to induce leukemia upon overexpression.[60] In addition, Mef2c does not affect serial replating activities[59] or cellular transformation[62] *in vitro*. In another *MLL*-AML mouse model, the *Irf8*$^{-/-}$ mice (which spontaneously develop myeloproliferation) were infected with murine leukemia virus to induce acute leukemia.[61] Leukemia was accompanied by viral integrations in several genes known to be involved in LSC development in *MLL*-rearranged AML, including *HoxA9*, *Meis1* and *Myb*. Integrations in *Mef2c* and the resulting ectopic *Mef2c* expression was found in $\sim$20% of myeloid tumors.[61] Ectopic Mef2c expression in Irf8-deficient bone marrow progenitors induces myeloid leukemia in recipient mice, so Mef2c acts as a cooperating oncogene in the Irf8-deficient leukemia model.[61] Mef2c also functions as a cooperating oncogene in Sox4-induced myeloid leukemia.[60] In the *MLL-ENL* AML mouse model, the absence of Mef2c (using *Mef2c*$^{f/-}$ $\times$ *Mx-Cre*$^{+}$ mice) does not affect the *MLL-ENL*-induced LSC establishment or maintenance. Instead, Mef2c may regulate leukemic cell migration and invasion as the Mef2c-target genes include chemokine receptors, chemokine ligands and matrix metalloproteinase genes that are involved in this process.[61] Therefore, the cooperative oncogenic effect of Mef2c can dictate the aggressiveness of myeloid leukemia.

High MEF2C expression has been found in MLL-rearranged AML patient samples.[59,63] In other established human myeloid leukemias, previously unrecognized MEF2C can be involved. For example, in AML, the RUNX1(AML1)–ETO fusion oncoprotein not only represses the myeloid transcription factor PU.1,[64,65] but also targets miR-223.[66] By recruiting chromatin remodeling enzymes to pre-miR-223, RUNX1-ETO hypermethylates a CpG-cluster in the vicinity of miR-223, which results in silencing of miR-223 and inhibition of myeloid differentiation. As Mef2c is directly repressed by miR-223,[53] silencing miR-223 leads to increased MEF2C expression, suggesting that MEF2C can have an important role in RUNX-ETO-induced myeloid differentiation arrest. Demethylating treatment with 5-azacytidine, RNA interference against RUNX1-ETO or ectopic miR-223 expression each increases the miR-223 level and restores myeloid differentiation in primary leukemic blasts from AML patients, but an effect on MEF2C expression levels was not reported.[66] Recently, in chronic myeloid leukemia, a link has been made between the fusion oncoprotein BCR–ABL, miR-223 downregulation and enhanced MEF2C expression.[67]

MEF2C IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

In pediatric T-cell ALL (T-ALL), distinct chromosomal rearrangements result in the activation of different oncogenes. Based on the expression of these oncogenes, genetic subgroups can be defined including the *TALLMO*, *HOXA*, *TLX3*/*HOX11L2* and *TLX1*/*HOX11* subgroups (reviewed in Meijerink[68]). Ectopic activation of these oncogenes facilitates differentiation arrest and cellular transformation at specific stages in T-cell development.[62,68,69] Cluster analyses on gene expression data of 117 primary T-ALL patient samples led to the identification of two additional T-ALL subtypes, each representing approximately 10% of all T-ALL cases.[62] Elaborate molecular and cytogenetic analyses revealed novel and unique oncogenic rearrangements in each of these subgroups. One of these novel subgroups has an arrest at the cortical or proliferative stage of thymocyte development and contains chromosomal rearrangements that lead to the ectopic expression of the homeobox transcription factor NKX2-1 or NKX2-2. The second novel subgroup is characterized by an early T-cell developmental arrest and various chromosomal rearrangements that result in the constitutive activation of *MEF2C*.[62] An independently identified immature subgroup based on the expression of early T-cell progenitor genes has been called early T-cell precursor (ETP) ALL.[70] The chromosomal translocations and deletions in the immature MEF2C T-ALL subgroup include the translocations *BCL11B-SPI1*, *BCL11B-NKX2-5*, *ETV6*(*TEL*)-*NCOA2*(*GRIP-1*) and *RUNX1*(*AML1*)-*AFF3*(*LAF4*), as well as a 5q14 deletion or an unbalanced translocation of the telomeric part of the chromosome upstream of *MEF2C*.[62] Each of these aberrations drives ectopic *MEF2C* expression, implicating MEF2C as the central oncogene in this subgroup. For example, the product of the *SPI1* gene, PU.1, and MEF2C are important factors in regulating lymphopoiesis and *MEF2C* is activated by PU.1 and/or both factors cooperate in a transcriptional complex.[49] NKX2–5 is not expressed in normal thymocytes and peripheral blood mononuclear cells,[71,72] but NKX2–5 (also a regulator of MEF2C in cardiac development[73]) is capable of driving *MEF2C* expression in T-ALL cell lines.[62,74] Furthermore, whole-genome sequencing of 12 ETP-ALL patient samples revealed a mutational spectrum resembling that of myeloid leukemias and includes inactivating mutations in *ETV6* and *RUNX1*,[75] but a link between these genes and MEF2C has not been reported.

Unlike the aberrantly expressed TLX1, TLX3 and NKX2-1/2-2 in the respective T-ALL subgroups, MEF2C is expressed during normal hematopoiesis. Strikingly, MEF2C is highly expressed in common lymphoid progenitors and in the B-cell compartment (Figure 3) and to date no MEF2C aberrations have been

documented in precursor-B ALL. However, a translocation resulting in the oncogenic fusion proteins MEF2D–DAZAP1 and DAZAP1–MEF2D has been reported in precursor-B ALL.[76–78] In addition, frequent mutations in the histone methyltransferase MLL2 and MEF2B (and less frequent MEF2C mutations) have been found in non-Hodgkin lymphoma.[79]

CONCLUDING REMARKS

As MEF2C is highly expressed in the CLP- and B-cell compartments (Figure 3) and to date no MEF2C aberrations have been documented in precursor-B ALL, we conclude that in normal development MEF2C helps to drive developing cells into the CLP lineage[49] and into the B-cell lineage, where MEF2C is functionally active.[55,56,80] This lineage direction can be a result of active transcription by cooperation of MEF2C with p300/CBP to acetylate histones (Figure 2). Alternatively, the lineage choice can be the default fate upon inhibition of another lineage choice. In this respect, MEF2C antagonizes the NOTCH1 (ICN) signaling activity (essential for T-lineage development) via competitive binding to MAML1. In fact, in ETP-ALL, characterized by an immature immunophenotype[70] and high ectopic MEF2C expression,[62] activating *NOTCH1* mutations have been reported to be less frequent when compared with the other more differentiated T-ALL genetic subgroups.[75,81] This suggests antagonism between NOTCH1- and MEF2C-active complexes—perhaps involving MAML1—in T-ALL. Therefore, ectopic expression of MEF2C in myeloid or early T-cell precursors, which normally have low or no MEF2C expression, can lead to a differentiation arrest and oncogenic transformation of these cell types and result in AML or ETP-ALL, respectively. MEF2C can be regarded as one of many transcription factors that are expressed as a remnant from earlier precursors/HSCs of which some have to be precisely down-regulated beyond the ETP stage (PU.1, C/EBPα, SCL/TAL, GATA-2) in order to allow T-cell development (reviewed in Rothenberg and Scripture-Adams[82]). The requirement of many transcriptional programs to be shut down in order for T-cell commitment to occur makes switching into different myeloid cell fates or transforming into T-ALL possible when one or more of these transcription factors fail to be inactivated. Contrary to the classic model (as depicted in Figure 3), substantial evidence indicates that in murine T-cell development, early T-cell precursors lose B lineage potential before losing myeloid lineage development potential.[83–86] With respect to this plasticity between the myeloid and ETP potential and depending on the cell context, aberrant MEF2C expression in an early progenitor could represent the onset of AML and ETP-ALL.

ABBREVIATIONS

MEF2, myocyte enhancer factor 2; MADS, MCM1-agamous-deficiens-serum response factor; bHLH, basic helix-loop-helix; MLP, muscle LIM-only protein; HDAC, histone deacetylase; MAML, Mastermind-like; ICN, intracellular NOTCH; MMP1, matrix metallo proteinase1; HSC, hematopoietic stem cell; CMP, common myeloid progenitor; GMP, granulocyte-monocyte/macrophage progenitor; MEP, megakaryocyte-erythrocyte progenitor; CLP, common lymphoid progenitor; BCR, B cell receptor; MPP, multipotent progenitor; LSC, leukemic stem cell; MLL, mixed lineage leukemia; AML, acute myeloid leukemia; T-ALL, T cell acute lymphoblastic leukemia; ETP-ALL, early T cell precursor acute lymphoblastic leukemia.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work is supported by the Children Cancer Free Foundation (Stichting Kinderen Kankervrij (KIKA), 2008-029 (KCB)).

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