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REVIEW

The multifaceted functions of C/EBPα in normal and malignant haematopoiesis

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The process of blood formation, haematopoiesis, depends upon a small number of haematopoietic stem cells (HSCs) that reside in the bone marrow. Differentiation of HSCs is characterised by decreased expression of genes associated with self-renewal accompanied by a stepwise activation of genes promoting differentiation. Lineage branching is further directed by groups of cooperating and counteracting genes forming complex networks of lineage-specific transcription factors. Imbalances in such networks can result in blockage of differentiation, lineage reprogramming and malignant transformation. CCAAT/enhancer-binding protein-α (C/EBPα) was originally identified 30 years ago as a transcription factor that binds both promoter and enhancer regions. Most of the early work focused on the role of C/EBPα in regulating transcriptional processes as well as on its functions in key differentiation processes during liver, adipogenic and haematopoietic development. Specifically, C/EBPα was shown to control differentiation by its ability to coordinate transcriptional output with cell cycle progression. Later, its role as an important tumour suppressor, mainly in acute myeloid leukaemia (AML), was recognised and has been the focus of intense studies by a number of investigators. More recent work has revisited the role of C/EBPα in normal haematopoiesis, especially its function in HSCs, and also started to provide more mechanistic insights into its role in normal and malignant haematopoiesis. In particular, the differential actions of C/EBPα isoforms, as well as its importance in chromatin remodelling and cellular reprogramming, are beginning to be elucidated. Finally, recent work has also shed light on the dichotomous function of C/EBPα in AML by demonstrating its ability to act as both a tumour suppressor and promoter. In the present review, we will summarise the current knowledge on the functions of C/EBPα during normal and malignant haematopoiesis with special emphasis on the recent work.

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INTRODUCTION

Proper haematopoietic differentiation requires a strict pattern of spatiotemporal gene transcription, which is orchestrated by intricate interactions of proteins with promoter and enhancer regions. In particular, binding of transcription factors to enhancer regions has been shown to facilitate the recruitment of RNA polymerase II to the target gene promoter and to induce epigenetic modifications that make the chromatin more accessible.[1] Work performed in embryonic stem cells has shown that transcription factors bind enhancers in a sequential manner and that embryonic stem cell-specific transcription factors establish an active epigenetic state at tissue-specific regulatory elements.[2] These factors are subsequently replaced by lineage-specific transcription factors that are able to induce the proper gene expression pattern of differentiating cells. Thus, dynamic binding of transcription factors appears to be mediated by the cell type-specific chromatin environment where extracellular cues are routed to intracellular transcription in a manner dependent on the recruitment of lineage-specific transcription factors to regulatory elements.

CCAAT/enhancer-binding protein-α (C/EBPα) is the founding member of a family of six transcription factors: C/EBP-α, -β, -γ, -δ, -ε and -ζ, which all share a basic region leucine zipper C-terminal domain. The C-terminal DNA-binding domain consists of an 86-residue α-helical structure with a leucine-rich hydrophobic part that allows for homo- or heterodimerization with family members and other proteins with a similar structure,[3,4] and simultaneously positions the basic region in the major groove enabling efficient binding to its cognate site on the DNA.[5,6]

CEBPA is an intronless gene located on chromosome 19q13.1 in humans. The single mRNA transcribed from the *CEBPA* gene is translated into two isoforms (Figure 1), due to alternative start site usage resulting in the full-length C/EBPα (42 kDa; p42) and a shorter, N-terminal truncated isoform (30 kDa; p30). P30 lacks the first 117 amino acids of full-length p42, which includes the transactivation domain 1 (TAD1). TAD1 has been shown to regulate transcriptional activation through interactions with components of the RNA polymerase II preinitiation complex, including the TATA-box-binding protein (TBP) and the transcription factor IIB.[7] TAD1 has also been implicated in C/EBPα-mediated repression of E2F activity, which has a role in the ability of C/EBPα to control cell cycle progression.[8–10] The ratio between p30 and p42 is regulated at the level of translation by eIF2a/eIF3E. Abundance of nutrients and growth factors results in the activation of eIF2a/eIF3E, increasing the p30/p42 ratio through the use of alternative translational start site usage. Thus, increased translation of p30 promotes proliferation in favourable conditions.[11]

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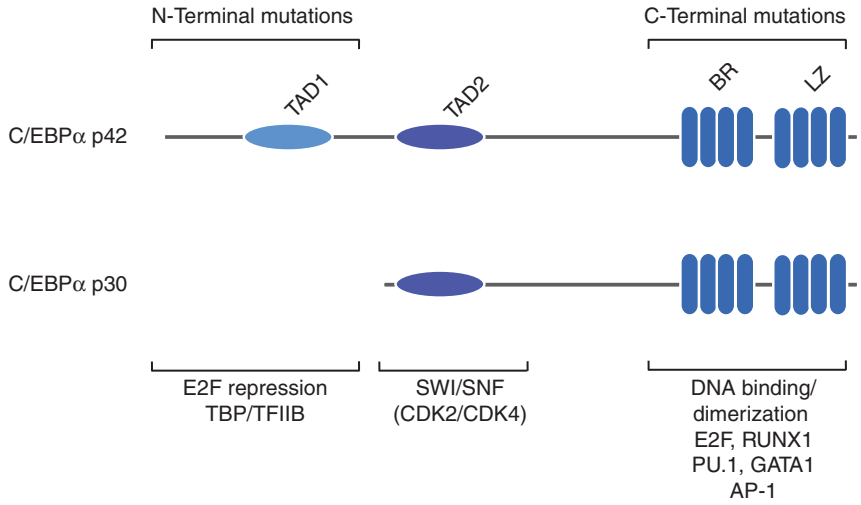

Figure 1. The functional domains of C/EBPα. The C-terminal basic region leucine zipper (BR-LZ) domain mediates DNA-binding and protein–protein interactions with transcription factors associated with differentiation and cell cycle control. Domains involved in transcriptional activation and E2F repression are lost in the truncated form of C/EBPα, whereas binding sites for proteins such as SWI/SNF and CDK2/CDK4 are retained in p30.

Both C/EBPα isoforms share the TAD2 that mediates antimitotic effects through direct interactions with p21[ref. 12] and regulates proliferation through interactions with the chromatin remodelling complex SWI/SNF.[13] In addition, proliferative arrest has been suggested to be mediated through inhibition of Cdk2/Cdk4.[14] However, more recent work has shown that deletion of the Cdk2/Cdk4-binding domains of *Cebpa* in mice has no effect neither on proliferation nor liver development.[15]

The transcriptional regulation mediated by C/EBPα is essential for proper differentiation of cells in various tissues. Wang *et al.*[16] were the first to demonstrate how non-conditional disruption of *Cebpa* affects liver development in the foetus. Thus, *Cebpa* deletion is perinatal lethal as a consequence of imbalanced energy homeostasis due to perturbed hepatic glycogenesis.[16] Furthermore, newborn *Cebpa*$^{-/-}$ mice exhibit impaired adipocyte maturation, accumulate immature myeloid cells and lack mature granulocytes.[17] In the haematopoietic system, C/EBPα is most prominently expressed in myeloid progenitor cells and its expression is subsequently downregulated as the cells differentiate (Figure 2). Hence, conditional deletion of *Cebpa* in the adult haematopoietic system of mice disrupts the transition between common myeloid progenitors and granulocyte monocyte progenitors (GMPs). Notably, although *Cebpa*$^{-/-}$mice accumulate immature myeloid progenitors, they do not develop acute myeloid leukaemia (AML).[18]

C/EBPA IN MYELOID DIFFERENTIATION

Myelopoiesis is the process by which myeloid progenitor cells differentiate into myeloid cells such as granulocytes (eosinophilic, basophilic and neutrophilic) and monocytes. The above-mentioned essential role of C/EBPα in this developmental process has spurred an extensive interest in deciphering the mechanisms by which C/EBPα mediates this process in cooperation with other myeloid transcription factors (reviewed in Friedman[19]).

Myeloid differentiation is primed at an early stage by PU.1[refs 20,21] and RUNX1[ref. 22] and is further directed by C/EBP family members.[17,18] Additional factors such as IRF8,[23] GFI1[ref. 24] and SCL/TAL1[ref. 25] have been shown to have a role in myeloid development. Terminal myeloid differentiation is partly mediated by extrinsic signalling through the cytokines granulocyte colony-stimulating factor and macrophage colony-stimulating factor, which instruct monocyte and granulocyte differentiation, respectively.[26]

The E-twenty-six- family member PU.1 is a transcription factor expressed in early progenitor cells and is required for the generation of both common myeloid progenitors and common lymphoid progenitors, as well as the terminal differentiation of macrophages and B cells. Interestingly, PU.1 possesses the rare ability of binding to tightly packed chromatin and DNA in nucleosome structures and induces changes in the chromatin conformation. Thus, PU.1 acts as a priming factor (pioneer factor) during haematopoietic maturation by generating cell-type-specific regions of open chromatin in *cis*-regulatory elements that serve as beacons for binding additional transcription factors and chromatin remodellers. Moreover, myeloid specification is mediated by colocalization of PU.1 with C/EBPα.[27] However, this effect appears to be dose-dependent as PU.1 and C/EBPα reciprocally regulate the expression of one another.[28–31] Specifically, C/EBPα enhances PU.1 expression, by directly binding to the *SPI1* promoter and the − 14 kb enhancer,[28,29] and represses PU.1 activity by displacing its cofactor c-Jun.[30] The PU.1:C/EBPα ratio has also been proposed to instruct terminal differentiation of myeloid progenitor cells. Indeed, inducible expression of PU.1 and C/EBPα in *Sfpi1*$^{-/-}$ cells (PU.1 deficient) shows that a high PU.1:C/EBPα ratio induces macrophage development, whereas a lower ratio directs the cells towards granulocytic maturation.[31] Furthermore, heterodimerization of C/EBPα with the AP-1 proteins c-Jun and c-Fos weakens the affinity for several canonical C/EBPα target genes while retaining specificity for PU.1, ultimately leading to skewing towards terminal monocytic differentiation.[3] Hence, C/EBPα appears to affect myeloid differentiation through several layers of regulation.

Studies aiming at investigating the specific function and regulation of C/EBPα during differentiation have been complicated by disparate results in different model systems. Studies of lineage determination of myeloid cells have primarily been performed in a bipotent myeloid leukaemic cell lines, and similar experiments performed *in vivo* have often shown diverging results. As an example, post-translational modifications of C/EBPα residues are thought to induce conformational changes of the protein that would alter the activity of the transactivation domain. However, whereas serine 248 phosphorylation was found to induce terminal granulocytic differentiation in myeloid cell lines,[32] substitution of serine 248 to alanine in knock-in mice does not affect terminal myeloid differentiation in young mice.[33] Similarly,

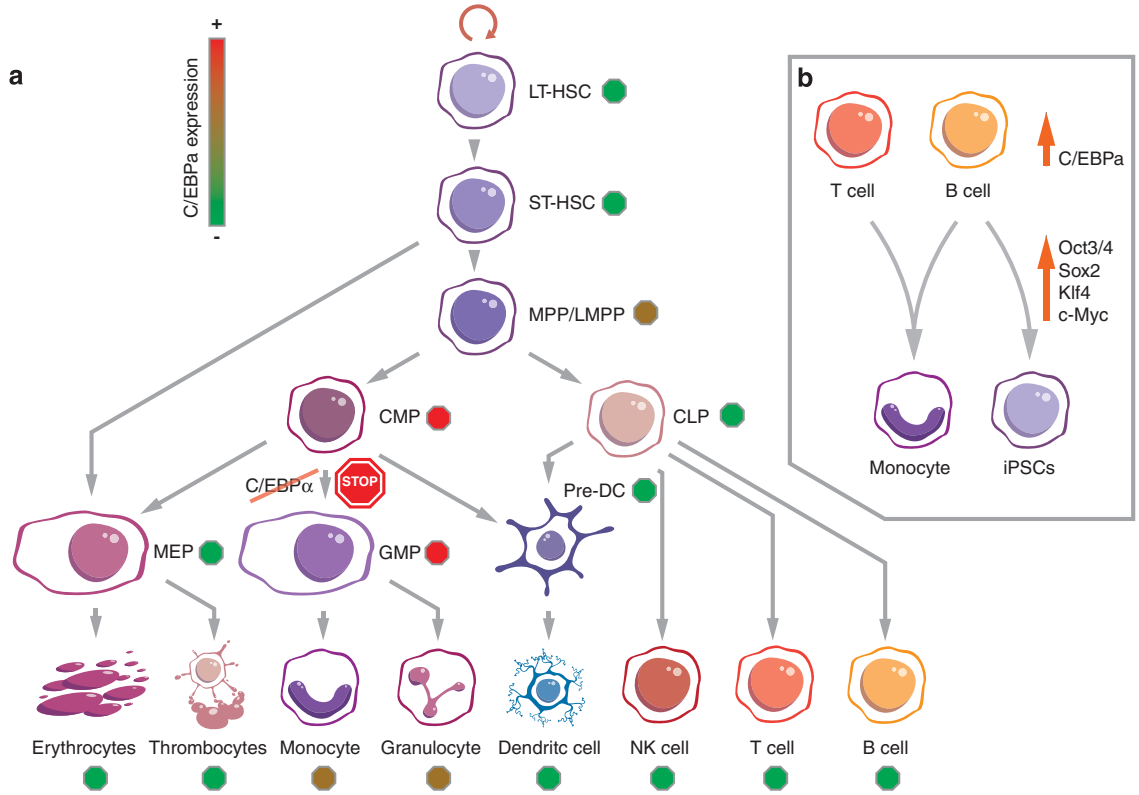

Figure 2. The role of C/EBPα in normal haematopoietic differentiation. (**a**) The haematopoietic hierarchy showing the expression of *Cebpa*. Expression data is indicated in colour (low green; red high) and derives from http://servers.binf.ku.dk/hemaexplorer/.[103] (**b**) The function of C/EBPα in cellular plasticity exemplified by the transdifferentiation of B/T cells towards monocytes induced by overexpression C/EBPα as well as by the enhancement of induced pluripotent stem cell (iPSC) generation, similarly mediated by overexpression C/EBPα (see main text for additional details).

exogenous C/EBPα directs granulocytic maturation of bipotent myeloid cell lines,[34] whereas ectopically expressed *Cebpa* in primary murine myeloid[35] or lymphoid[36,37] progenitor cells induce monocytic maturation. Collectively, differentiation is a complex process, dependent on an intricate network of intrinsic and extrinsic factors, and there is a need for caution when drawing conclusions solely based on work performed in one model system. Thus, the ability of C/EBPα to drive context-specific differentiation of cells in diverse tissues is not only a matter of proper dosing but also depends upon the collaborating actions of additional transcription factors.

NOVEL FUNCTIONS FOR C/EBPA IN HSCS

Although C/EBPα is mainly considered a lineage-instructive factor crucial for myeloid differentiation, recent data has shown that it also has an important role in haematopoietic stem cells (HSCs). HSCs exhibits a low but robust expression of *Cebpa*, and consistently *Cebpa*$^{-/-}$ mice display HSC phenotypes. Specifically, the Tenen Lab reported that the conditional loss of *Cebpa* in adult HSCs leads to an immediate expansion of functional HSCs, which was associated with an increase in proliferation.[38] Further, it was shown that *Cebpa*$^{-/-}$ HSCs upregulated a foetal HSC gene expression programme as well as N-Myc, and that the latter was responsible for the increase in HSC proliferation. Using a similar system, i.e. *Mx1-Cre* mediated deletion of *Cebpa*, we recently showed that C/EBPα deletion was associated with a dramatic loss in HSC self-renewal.[39] In fact, *Cebpa*$^{-/-}$ HSCs were lost in secondary recipients and *Cebpa*$^{-/-}$ bone marrow donor cells failed to rescue irradiated mice in non-competitive serial transplantation experiments. Further, we were able to show that *Cebpa*$^{-/-}$ HSCs displayed a marked increase in markers of DNA damage and apoptosis. Finally, using chromatin immunoprecipitation and sequencing analysis in HSCs and multipotent progenitor cells, we demonstrated that C/EBPα bound to genes destined for expression later during myeloid differentiation. As these regions are considered inaccessible in HSC/multipotent progenitor cells, this could potentially reflect the ability of C/EBPα to function as a pioneer factor.

Recently developed *Cebpa* reporter mouse lines, driven by the entire endogenous *Cebpa* regulatory regions or a +37 kb myeloid-specific enhancer, have also been informative with respect to the function of C/EBPα in HSCs.[40,41] In both lines, only a fraction of long-term HSCs express *Cebpa* (4–20%), suggesting that *Cebpa* expression marks a minor subset of LT-HSCs. Interestingly, one of these reports demonstrated that essentially all reconstituting activity was found in the *Cebpa*-expressing compartment, suggesting that C/EBPα is indeed essential for long-term HSC function, at least during haematopoietic reconstitution.[40]

The above-mentioned discrepancies between the *Cebpa*$^{-/-}$ phenotypes reported by our group and the Tenen group may reflect, in part, differences in the timing of analysis relative to the polyinosinic:polycytidylic acid-mediated activation of the Mx1-Cre driver. Specifically, because of the earlier time point generally used in the Tenen report, their analyses are focused on the effects of *Cebpa* deletion in actively cycling HSCs as a consequence of the impact of interferon signalling on HSCs.[42] Indeed, loss of *Cebpa* in this scenario may convert adult HSCs to a more foetal-like state as suggested by the Tenen group.[38] In any event, both reports clearly demonstrate the importance of intact C/EBPα function for the proper control of HSC numbers and functions.

C/EBPA LEVELS OR FUNCTION IS PERTURBED IN HUMAN AML

AML is a clonal disorder that arises through the acquisition of genetic and epigenetic alterations ultimately leading to changes in the transcriptional wiring of the leukaemic cells and/or loss of cell identity. In line with this, mutations in transcription factors and epigenetic regulators with roles in normal haematopoietic development are among the most frequent aberrations detected in human AML patients.[43,44]

Mutations in *CEBPA* in human AML were first reported by Pabst *et al.*[45] and have later been described in numerous studies.[46–55] *CEBPA* mutations mainly fall into two classes. The first class involves the C-terminal part of C/EBPα, and consistently these mutations interfere with the DNA-binding and/or dimerisation properties of the protein.[56,57] The second class is located in the 5′ end of the gene and frequently disturbs the open reading frame. Most of these mutations reside between the two ATGs initiating the respective synthesis of the p42 and p30 isoforms, and, consequently, lead to the exclusive expression of the p30 isoform from the affected allele.

CEBPA mutations in human AML have been shown to be either mono- or biallelic. Monoallelic *CEBPA* mutations are associated with a plethora of other genetic lesions and are generally heterogeneous with respect to gene expression and prognosis.[49,58–60] In contrast, biallelic *CEBPA* mutant AML constitutes a distinct subtype associated with good prognosis and was recognised as such in the recent World Health Organisation classification.[61] Further support for this comes from gene expression studies, which also classify biallelic *CEBPA* mutant AML as a distinct disease entity.[48,49,58–60,62] Biallelic *CEBPA* mutant AMLs preferentially combine an N-terminal mutation on one allele (sustaining the expression of p30 only) with a C-terminal mutation on the other (deficient in dimerization/DNA binding).[54] As C/EBPα functions as a dimer, this implies that the only C/EBPα dimers able to bind DNA will be p30/p30 homodimers in AML with this combination of mutations. The remaining biallelic *CEBPA* mutant AMLs either combine two N-terminal mutations or an N-terminal mutation with a frameshift/nonsense mutation in the central part of *CEBPA*. As the latter mutations encode C/EBPα variants lacking the basic region leucine zipper domain, these combinations will result in the formation of p30/p30 homodimers as the only C/EBPα entities capable of binding DNA. Hence, these findings underscore the importance of both the presence of the p30 isoform and lack of functional p42 isoform in *CEBPA* mutant AML (Figure 3).

Biallelic *CEBPA* mutant AML exhibits a distinct secondary mutational spectrum that sets this class aside from other AML subtypes.[54] Although this subtype frequently exhibits mutations in well-known leukaemic players such as *ASXL1*, *RUNX1* and *TET2*, these events are clearly under-represented when compared with other AML subtypes. On the other hand, mutations in *WT1* and, in particular, *GATA2* are highly enriched in biallelic *CEBPA* mutant AML, suggesting a specific collaboration between these lesions and mutated *CEBPA*.[54] Interestingly, this pattern is also recapitulated in the rare cases of familial *CEBPA* mutant AML involving families of heterozygous carriers of N-terminal *CEBPA* mutations.[63] Here, progression to AML is associated with acquisition of a C-terminal mutation on the remaining *CEBPA* allele as well as with frequent lesions in *GATA2* or *WT1*.[64] Collectively, these findings demonstrate that biallelic *CEBPA* mutant AML constitutes a distinct AML entity.

The involvement of C/EBPα in human AML is not restricted to *CEBPA* mutant AML. Methylation of the distal *CEBPA* promoter was shown to correlate inversely with the expression levels of *CEBPA*, but did not have any prognostic value in normal karyotype AML.[65] However, the finding that *CEBPA* promoter methylation and

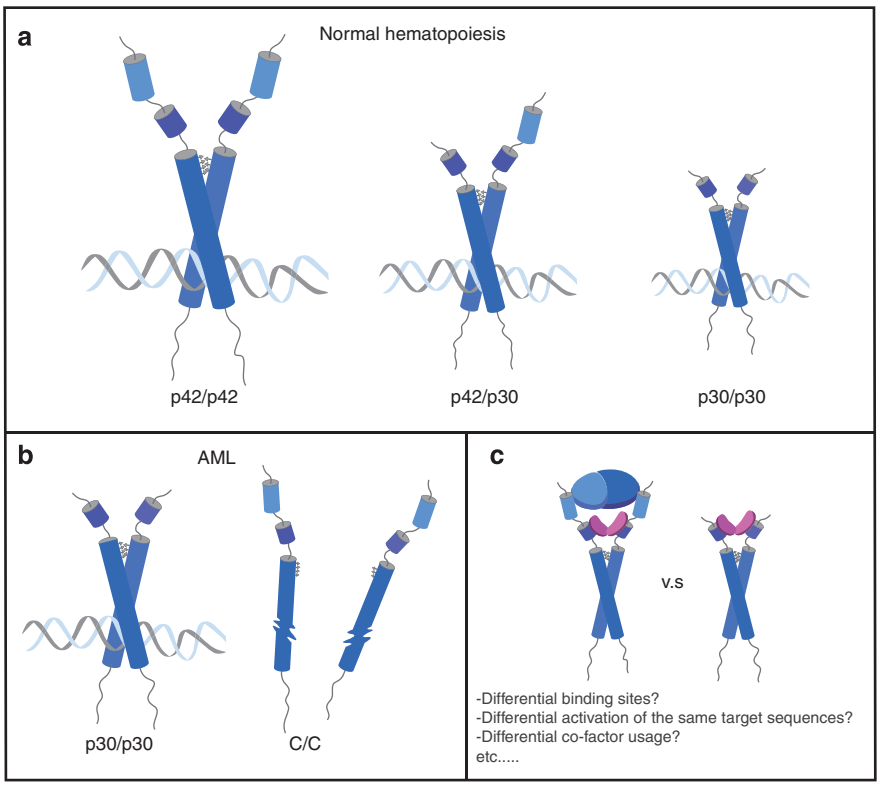

Figure 3. Differential actions of the p42 and p30 isoforms of C/EBPα. (**a**) Generally, the p42 isoform is more abundant than the p30 isoform in normal cells. Hence, the most prevalent C/EBPα entities are p42/p42 homodimers (as indicated by size). The presence of the TAD1 domain allows this isoform to interact with, and repress the activity of, E2F family members, which is key to its ability to repress proliferation. (**b**) In biallelic *CEBPA* mutant AML, the selective loss of functional p42 leads to the preferential formation of p30/p30 homodimers. (**c**) The potential molecular consequences of p42/p42 or p30/p30 expression (see main text for additional details).

mutations in the coding region of *CEBPA* are mutually exclusive implies that these events perturb similar cellular pathways. This is reinforced by the clustering of the transcriptional profiles derived from human *CEBPA* silent and biallelic mutant AML.[66]

Oncogenic driver proteins have also frequently been implicated in downregulating the expression of *CEBPA* in AML. One example is AML-ETO (t(8;21)), which downregulates the expression of *CEBPA*, most likely by interfering with an autoregulatory loop sustaining its expression.[67,68] At the post-transcriptional level, RNA-binding proteins such as hnRNP E2 and calreticulin expressed in blast crisis chronic myeloid leukaemia and AML1-MDS1-EVI (t(3;21))/ -MYH11 (inv16) in AML, respectively, have been shown to interfere with the translation of the *CEBPA* mRNA.[69–72] Finally, a number of oncogenic lesions also interfere with the function/levels of the C/EBPα protein (reviewed in Mueller and Pabst[73]). One particularly interesting case is the role of *TRIB2*, which was found to be overexpressed in a distinct subtype of AML frequently harbouring mutations in *NOTCH1*.[74,75] *TRIB2* is a potent oncogene and its overexpression in mouse haematopoietic stem and progenitor cells results in AML. Gene expression analysis further revealed that the *TRIB2*-high human AML clustered with biallelic *CEBPA* mutant AML, suggesting that they operate in similar pathways.[75] Indeed, TRIB2 was found to mediate the degradation of the p42 isoform of C/EBPα leading to a skewed ratio of p42/p30, resulting in the preferential formation of p30/p30 homodimers. This, in turn, explains the resemblance between biallelic *CEBPA* mutant and *TRIB2*-high AML.[74,76] Collectively, these findings demonstrate that a large fraction of human AMLs converges at downregulating the level and/or interferes with the function of C/EBPα, thereby unambiguously classifying it as a key myeloid tumour.

THE REQUIREMENT FOR C/EBPA IN AML DEVELOPMENT

Despite the numerous pathways by which C/EBPα levels and functions are perturbed in human AML, cases with a complete lack of C/EBPα have yet to be reported.[77] This is consistent with the lack of leukaemia development in *Cebpa*$^{-/-}$ mice and suggests that in addition to its well-established tumour-suppressive function, C/EBPα may actually also be required for the development of AML.[78] Consistently, forced expression of the fusion oncogene *BCR-ABL* in *Cebpa*-deficient murine foetal liver cells results in erythroleukaemia instead of the chronic myeloid leukaemia, which normally arises in this setting.[79] These findings formed the basis of a model where the block in myeloid differentiation upstream of the GMP leads to a failure of *Cebpa*$^{-/-}$ progenitors to reach a stage of myeloid identity, which may be a prerequisite of leukaemic transformation.

We have recently put this model to the test by assessing the requirement of C/EBPα in MLL-ENL-mediated transformation.[80] Here we find that C/EBPα is absolutely required for the initiation of MLL fusion-driven AML and that even deletion of *Cebpa* at the GMP stage completely abrogates transformation. These findings suggest that it is not the lack of myeloid identity *per se* that underlies the requirement for C/EBPα in AML development, but more likely its ability to collaborate with MLL-ENL (and perhaps other fusion oncogenes/transcriptional regulators) in initiating the expression of a transcriptional programme required for leukaemic transformation. Strikingly, the deletion of C/EBPα in already established MLL-ENL-driven leukaemias had absolutely no impact in terms of the expression of this transcriptional programme and/or the properties of the leukaemic cells.

The demonstrated requirement for C/EBPα in the development of myeloid leukaemia was recently revisited.[81] Consistent with the findings detailed above, leukaemic transformation mediated by MLL-AF9 was found to be strictly dependent on the presence of C/EBPα, whereas already established AMLs were refractory to C/EBPα loss. Interestingly, when mice transplanted with MLL-AF9-expressing *Cebpa*$^{-/-}$ progenitors were subjected to hydrodynamic injection of interleukin-3 and granulocyte–macrophage colony-stimulating factor-encoding vectors, *Cebpa*$^{-/-}$ AML readily developed. As interleukin-3 and granulocyte–macrophage colony-stimulating factor mediate formation of GMPs in a C/EBPα-independent manner (in a process termed emergency granulopoiesis[82]), the authors concluded that leukaemic transformation was dependent on the formation of GMPs and not C/EBPα *per se*. However, as emergency granulopoiesis upregulates the expression of *Cebpb* and is abrogated by *Cebpb* knockdown, it is likely that C/EBPβ may functionally substitute C/EBPα as has indeed been reported earlier.[82,83] Also, the report fails to explain why *Cebpa*$^{-/-}$ GMPs are refractory to leukaemic transformation.[80]

The findings reported above raise the possibility that C/EBPα may act as a so-called pioneer factor that facilitates access/remodelling at key regulatory elements, thereby enabling access of other factors such as MLL-ENL. Once these elements are remodelled, C/EBPα is no longer required for the function of these regulatory elements, consistent with the lack of phenotype of removing C/EBPα in already established AML.[80] Consistent with this notion, C/EBPα has been reported to collaborate with HOXA9 in HOXA9-dependent AML.[84] The function of C/EBPα as a potential pioneer factor also appears to extend to non-haematopoietic systems, specifically adipocyte differentiation, where C/EBPα was demonstrated to facilitate the binding of the adipogenic master regulator peroxisome proliferator-activated receptor-γ to compacted chromatin.[85] This property of C/EBPα was dependent on its SWI/SNF interaction domain, which has also previously been found to be essential for C/EBPα-mediated myeloid differentiation.[86]

Collectively, several lines of evidence suggest that, in addition to its tumour-suppressive function, C/EBPα is also required for the development of AML, most likely through its ability to collaborate with other transcriptional regulators and by promoting chromatin remodelling.

THE PROMOTION OF LEUKAEMIC TRANSFORMATION BY MUTATIONS IN *CEBPA*

Mutations in *CEBPA* are associated with leukaemic transformation, but how does these mutations mediate their effect? Mouse models have been instrumental in the efforts to answer this question, and the first *Cebpa* mutant lines, where an AML-like disease was reported, harboured mutations in the basic region of C/EBPα. Specifically, these mutations abrogated the interactions with members of the E2F family of cell cycle regulators without interfering with the ability of C/EBPα to bind DNA.[9] These animals developed a number of conditions ranging from neutropenia, over myeloproliferation to an AML-like syndrome with limited peripheral involvement, presumably mediated by the acquisition of secondary mutations.[87] Although similar mutations were not identified in human AML (in the patient studies that followed), these findings underlined the importance of C/EBPα-mediated repression of E2F activity in protecting against aberrant myelopoiesis.

As discussed above, the most common group of *CEBPA* mutations in human AML leads to the expression of the p30 isoform of C/EBPα. Consistently, knock-in mice homozygous for an allele of *Cebpa* that exclusively express this isoform (*Cebpa*$^{N/N}$) all succumb to full-blown AML within the first year of their lives.[88] Remarkably, but consistent with data from retroviral transduction models, the leukaemic stem cells in these models are residing in a population of committed progenitors,[89] thus challenging the previous dogma that leukaemic stem cell were residing in the HSC compartment.[90] Indeed, recent efforts characterizing the cellular compartments that harbour oncogenic driver mutations in human AML have confirmed that the cell-of-origin, and consequently the leukaemic stem cell, is residing in populations downstream of HSCs.[91,92]

Following the characterisation of the *Cebpa*$^{N/N}$ strain, the Nerlov group developed a mouse line where they combined the *Cebpa*^{N} allele with an allele expressing a C-terminal mutation unable to bind DNA (*Cebpa*^{C}), thereby mimicking the most frequent combination of mutations in *CEBPA* mutant AML.[93] The N/C combination promoted leukaemic development with a shortened latency as compared with the N/N combination, and this appeared to be conferred by an expansion of the HSC compartment in the preleukaemic state. Such an expansion may favour the acquisition of secondary mutations in downstream populations, thus leading to a faster developing AML.

As discussed above, both the N/N and the N/C combinations are predicted to lead to the formation of p30/p30 homodimers as the only C/EBPα entity capable of binding DNA. So what underlie the differences in latencies between N/N and N/C murine leukaemias? One potential explanation could be that, although the C-terminally mutated C/EBP variants are unable to bind DNA, they may still bind to C/EBP-interacting proteins such as PU.1 and/or SWI/SNF complexes.[94] Thus, the expression of the *Cebpa*^{C} allele could potentially reduce the amount of C/EBP activity in the cell, thereby reducing the ability of other C/EBPs to rescue the effects of expressing the aberrant C/EBPα variants.

Recently, a more advanced model of *CEBPA* mutant AML was constructed by combining the *Cebpa*$^{N/C}$ mice with mutations in tyrosine kinase *FLT3*.[95] *FLT3* mutations are frequent in human AML and are found in combination with biallelic *CEBPA* mutations, although not enriched in this subtype.[54] Consistent with its leukaemogenic role, *FLT3* mutations further accelerate disease development in *Cebpa*$^{N/C}$ mice, underlining the importance of developing more refined models of *CEBPA* mutant AML. In particular, it would be interesting to combine the current model with mutations in *GATA2* and *WT1*, both of which are particularly enriched among biallelic *CEBPA* mutant cases.[54]

Both mouse and patient studies clearly indicate a central role for the p30 form of C/EBPα in the development of AML and suggest that the formation of p30/p30 homodimers are crucial in mediating oncogenic transformation. It is well established that loss of C/EBPα-mediated E2F cell cycle control, either by the expression of basic region mutant/p30 isoforms of C/EBPα or through its complete loss, leads to a block in myeloid differentiation. However, the differences in the ability of these genetic aberrations to support leukaemic development suggest that the role of C/EBPα in cell cycle progression is not the only factor governing the tumour suppressor functions of WT C/EBPα.

Being a transcription factor, it is likely that the normal gene regulatory properties of WT C/EBPα are affected not only by the complete loss of all C/EBPα isoforms but also by the preferential expression of p30/p30 homodimers that is observed in AML. Loss of C/EBPα was recently shown to lead to the upregulation of *Cebpg*, both in *CEBPA*-silenced AML and in murine *Cebpa*$^{-/-}$ stem and progenitor cells.[96] Interestingly, knockdown of *Cebpg* in both these systems promoted neutrophilic differentiation demonstrating that C/EBPα-mediated repression of *Cebpg* has a role in the differentiation arrest induced by its loss in both *CEBPA*-silenced AML and during normal myeloid differentiation. Moreover, C/EBPα has also been shown to repress the expression of *Sox4*, a supposed oncogene in several cancer types.[66] Strikingly, not only did knockdown of *Sox4* inhibit the increased self-renewal and lack of myeloid differentiation observed in *Cebpa*$^{-/-}$ murine stem and progenitors but it also extinguished the self-renewal of leukaemic stem cells derived from murine *Cebpa*$^{N/C}$ AML. Consistent with these findings, *Sox4* expression was found to be upregulated in both human *CEBPA*-silenced AML and in human *CEBPA* mutant AML. Finally, overexpression of *Cebpa*^{C} in a bone marrow transplantation model has recently been shown to repress the expression of *Csf1r*.[97] However, a potential tumour suppressor function of *Csf1r* was not supported by its overexpression in the *Cebpa*^{C} overexpression setting. On the contrary, accelerated AML development was observed. The extent to which this reflects this particular model is not clear, but the potential functional interaction between C/EBPα and CSF1R in AML warrants further investigation.

These examples aside, we know very little about global differences between p42/p42- and p30/p30-mediated transcriptional regulation, and a challenge for the future will be to address this issue using genome-wide approaches. Specifically, are we able to find genes that are deregulated through loss of p42 binding, and/or does p30 bind to sites that are also occupied by p42 in normal progenitors? Or do p42 and p30 generally share the same binding patterns, only differing in their ability to control the expression of the cognate genes? Indeed, given the recent demonstration of the selective binding of p30/p30 dimers to WDR5, differential cofactor binding of the two C/EBPα isoforms should be investigated further.[98] Such approaches will lead to the identification of genes controlling leukaemic properties and potentially to genes that could be targeted therapeutically.

C/EBPA AS A PIONEER FACTOR AND A REGULATOR OF CELL IDENTITY

Throughout this review, we have described several instances alluding to the ability of C/EBPα to drive rearrangements of closed chromatin (Figure 4). These examples include the binding of C/EBPα to myeloid lineage-affiliated genes in HSCs/multipotent progenitor cells, the ability of C/EBPα to mediate the binding of peroxisome proliferator-activated receptor-γ to compacted chromatin in mouse embryonic fibroblasts and the requirement for C/EBPα for MLL fusion proteins to access chromatin during AML development.[39,80,85] Moreover, C/EBPα has been found to mediate robust transdifferentiation of both T and B cells into macrophages, that is, a process involving major rearrangements of both chromatin and gene expression patterns.[36,99–101] However, most strikingly, C/EBPα has been shown to enhance (>100-fold) the ability of the four Yamanaka factors to reprogramme B cells to induce pluripotent stem cells in a process mediated, in part, by the epigenetic regulator TET2.[100,102]

Collectively, these findings suggest that C/EBPα possesses some unique properties allowing it to interact with compacted chromatin in processes with importance for normal differentiation and for the development of cancer. Molecularly, these properties have been associated with its ability to recruit SWI/SNF complexes and to induce the expression of TET2. A task for the future will be to understand the underlying mechanisms of C/EBPα-mediated chromatin remodelling in the different settings outlined above.

CONCLUDING REMARKS

Here we have reviewed the current knowledge on C/EBPα in normal and malignant haematopoiesis. Strikingly, whereas the p42 isoform of this apparently uncomplicated transcription factor acts as a tumour suppressor in AML, it is equally required for the development of at least one subtype of this disease. The relative balance between context-dependent actions of C/EBPα in AML should be addressed in future studies. An equally important question to address is how the truncated p30 isoform of C/EBPα promotes AML. Does it bind selectively to some target genes, does it interact differently with various cofactors and/or does it perturb the functions of other bZIP transcription factors by heterodimerization? Finally, the exact mechanisms by which C/EBPα functions in HSCs and during myeloid differentiation still need to be fully established. Addressing all these questions has the potential of uncovering the mechanisms by which C/EBPα governs AML development and may thus potentially uncover novel targets for therapeutic intervention in AML with C/EBPα involvement.

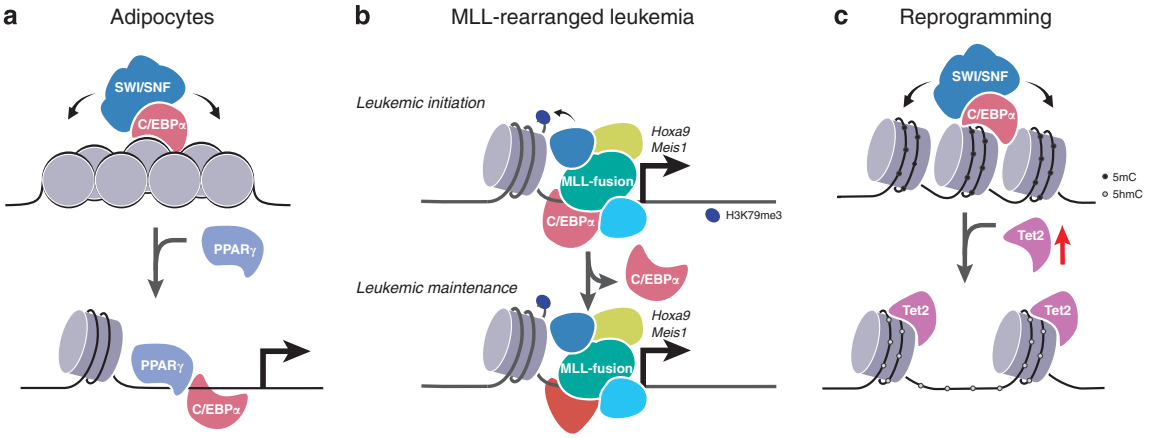

Figure 4. C/EBPα as a pioneer factor. (**a**) C/EBPα has the potential to remodel chromatin through its ability of interacting with members of the SWI/SNF complexes.[86] During adipose differentiation, this modelling allows for binding of another key adipogenic factor peroxisome proliferator-activated receptor (PPARγ).[85] (**b**) C/EBPα is also required for MLL-ENL-mediated transformation to AML, presumably through its ability to recruit MLL-ENL to specific genomic loci.[80] (**c**) Enforced expression of C/EBPα greatly enhances the reprogramming of haematopoietic progenitor cells to induced pluripotent stem cells (iPSCs). Presumably, C/EBPα binds to and facilitates the opening of loci, which are normally inaccessible in these progenitor cells. In addition, C/EBPα enhances the expression of TET2 leading to further transcriptional derepression through TET2-mediated hydromethylation.[100,102]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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