Macrophage migration inhibitory factor is overproduced through EGR1 in TET2<sub>low</sub> resting monocytes

Somatic mutation in TET2 gene is one of the most common clonal genetic events detected in age-related clonal hematopoiesis as well as in chronic myelomonocytic leukemia (CMML). In addition to being a pre-malignant state, TET2 mutated clones are associated with an increased risk of death from cardiovascular disease, which could involve cytokine/chemokine overproduction by monocytic cells. Here, we show in mice and in human cells that, in the absence of any inflammatory challenge, TET2 downregulation promotes the production of MIF (macrophage migration inhibitory factor), a pivotal mediator of atherosclerotic lesion formation. In healthy monocytes, TET2 is recruited to MIF promoter and interacts with the transcription factor EGR1 and histone deacetylases. Disruption of these interactions as a consequence of TET2-decreased expression favors EGR1-driven transcription of MIF gene and its secretion. MIF favors monocytic differentiation of myeloid progenitors. These results designate MIF as a chronically overproduced chemokine and a potential therapeutic target in patients with clonal TET2 downregulation in myeloid cells.
Ten-eleven-translocation (TET) proteins are iron-[Fe(II)]- and α-ketoglutarate (α-KG)-dependent dioxygenases that promote active DNA demethylation through iterative oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxycytosine (5caC), eventually leading to the replacement of 5mC by native C1. 5hmC oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) also prevents DNA methylation by decreasing cytosine accessibility to DNA methyltransferases. This activity of TET enzymes is regulated via substrate and cofactor availability, post-transcriptional regulation, and post-translational modifications.

TET family member interacting proteins may tether them to DNA. For example, TET2 could interact with NANOG in mouse embryonic stem cells, SP1/PU.1 in differentiating B-cells and monocytes, early growth response 2 (EGR2) in IL4/GM-CSF-driven human monocyte differentiation and Wilms tumor suppressor gene 1 (WT1) in acute myeloid leukemia cells. The binding of TET proteins to 5mC-free promoters with diverse partners suggested that they could function independently of their catalytic activity. In brain epigenome programming during postnatal development, EGR1 recruits TET1 to demethylate EGR1 binding sites. TET1 regulates gene transcription in mouse embryonic stem cells through association with the Sin3A corepressor complex and MOF histone acetyltransferase, while a catalytically dead Tet2 mutant represses Interleukin-6 (IL6) gene transcription in mouse macrophages by recruiting Hdac1 and Hdac2 histone deacetylases. Some of the catalytic-activity independent effects of TET proteins are mediated by the recruitment of OTG [O-linked N-acetylglucosamine (O-GlcNAc) transferase] to gene promoters.

Mono- or bi-allelic somatic mutations along the entire coding TET2 region are recurrent events in human hematopoietic malignancies, especially in chronic myelomonocytic leukemia (CML) in which mono- or bi-allelic mutations in TET2 gene are detected in 57% of patients. Mouse models with Tet2 gene deletion in hematopoietic stem cells develop a myeloid or a lymphoid malignancy. The long latency and low penetrance of these diseases suggest that cooperation with another genetic event and/or a permissive environment may be needed for malignancy emergence.

Somatic mutation in TET2 gene is also one of the most common clonal genetic events detected in the peripheral blood of ageing healthy individuals, named Clonal Hematopoiesis of Indeterminate Potential (CHIP). These TET2-mutated clones can be a first step towards a malignancy such as CMMI. A TET2-mutated CHIP also increases the risk of death from cardiovascular disease. A deregulated production of inflammatory cytokines by mutated myeloid cells may explain the cardiovascular risk associated with TET2 CHIP as these cytokines may promote leukocyte recruitment to atherosclerotic plaques. Accordingly, Tet2-deletion in murine macrophages induces a constitutive expression of lipopolysaccharide (LPS)-induced genes and cardiovascular risk is reduced on a genetic background of the Tet2 gene.

Here, we show that, in the absence of any inflammatory challenge, TET2 gene downregulation induces an overproduction and secretion of MIF (macrophage migration inhibitory factor). Initially identified as a lymphocyte-derived soluble product and released by a variety of cells and behaves as a proinflammatory cytokine with pathogenic roles in inflammatory and autoimmune disorders. Genetic deletion of MIF impairs the production of inflammatory mediators by monocytes/macrophages and prevents the inflammasome activation. We show that in CMLM human monocytes harboring truncating variants of TET2 gene, MIF secretion is increased through EGR-1 transcription factor recruitment to its promoter. These results identify MIF as a potential therapeutic target to prevent atherosclerosis and progression of TET2 mutated CHIP towards a chronic myeloid malignancy.

**Results**

**TET2 downregulation induces MIF overproduction.** We investigated whether TET2 gene downregulation could alter cytokine secretion by myeloid cells. Human cord blood CD34+ cells, transduced with TET2 or scrambled (SCR) shRNA lentiviruses, were sorted and cultured with SCF, IL-3, TPO, and GM-CSF to promote granulocytic/monocytic differentiation. Using cytokine-arrays to analyze day-10 cell culture supernatant, three cytokines were readily detected: MIF, G-CSF, and IL-1RA. MIF was repeatedly increased, while G-CSF and IL-1RA were not affected when TET2 expression was decreased (Fig. 1a, b). ELISA measurements confirmed MIF overproduction upon TET2 silencing at days 8–10 of culture (Fig. 1c). Consistent with these observations, MIF mRNA (Fig. 1d) and secreted MIF (Fig. 1e, f) were increased in four human leukemia cell lines (kasmu-1, M07e, UT-7, and TF-1) in which TET2 gene expression was decreased by using lentiviral shRNA (Supplementary Fig. 1a), as previously described. Finally, MIF concentrations were increased in blood (Fig. 1g) and supernatant of bone marrow aspirations (Supplementary Fig. 1b) of two Tet2-deficient mouse models. In these models, MIF plasma levels increased in mice 1–3 aged months, i.e., before changes in white blood cell count (Supplementary Fig. 1c, d), thus monocyte counts in the peripheral blood as a confounding factor. These results indicated that, in vitro and in vivo, in mouse and human cells, TET2 downregulation increased MIF secretion.

**MIF is overexpressed in TET2-mutated CMLM monocytes.** CMMI is a clonal disorder, mostly observed in the elderly with a median age at diagnosis of 73 years. TET2 mutations are early somatic events and the most frequent genetic alteration of this disease. To explore the link between TET2 mutations and MIF expression in human primary samples, we sorted peripheral blood monocytes from 60 CMMI patients (17 TET2-wild-type [TET2WT], 23 TET2-mutated [TET2MUT]) cases (Supplementary Table 1) and from 10 age-matched healthy donors and performed bulk RNA sequencing. Focusing on cytokine genes, IL6 mRNA was not expressed in control and CMMI resting monocytes while a significant increase in MIF mRNA was observed in TET2MUT compared to healthy donors and TET2WT CMMI (Fig. 2a; Supplementary Data 1). We also observed an inverse correlation between TET2 and MIF mRNA expression levels in CMMI patient monocytes (Fig. 2b). MIF increased mRNA detected in TET2MUT monocytes was validated by RT-qPCR analysis in an independent cohort of 146 CMMI patients (56 TET2WT and 90 TET2MUT cases including 68 truncating variants) compared to 19 young and 8 age-matched healthy donors (Supplementary Table 2 and Fig. 2c). Importantly, we did not detect any significant recurrent change in the expression of other cytokine-encoding genes in TET2-mutated patient monocytes, i.e., MIF gene expression was frequently increased in cells expressing truncating TET2 variant whereas the increased expression of other cytokine-encoding genes was heterogeneous and independent of TET2 status (Fig. 2d).

In a multi-institutional study (n = 1084 CMMI patients), we recently reported an overall survival advantage associated with TET2 mutations in CMMI patients. This was especially significant in the context of multiple or truncating TET2 mutants (trTET2MUT), i.e., nonsense and frameshift variants, compared to
non-truncated variants (non-trTET2MUT), i.e., in frame insertion/deletion, missense, and splice site variants. From our RNA sequencing data, the decreased expression of TET2 gene observed in trTET2MUT cells correlated with an increased expression of MIF mRNA, which was not detected in non-trTET2MUT cases (Fig. 2e). Among CMML samples tested by RT-qPCR (Fig. 2c), we observed an increased expression of MIF gene in trTET2MUT samples only (Fig. 2f). The previously described better survival of TET2MUT CMML patients was validated in the present cohort (Supplementary Fig. 2a) with no
**Fig. 1** TET2 downregulation promotes MIF production. a, b Cytokine profile arrays of supernatant collected from cord blood CD34+ cells infected with SCR- and TET2-shRNA-GFP cells infected with SCR- and TET2-shRNA-GFP, sorted on GFP expression, and induced to differentiate with stem cell factor (SCF), interleukin-3 (IL-3), Fms-related tyrosine kinase 3 ligand (FLT3L) and granulocyte-colony stimulating factor (G-CSF). a Representative cytokine array with supernatants collected at day 10 of differentiation. The rectangle points to MIF detection. b Quantification of MIF signals, normalized to positive controls. Data are mean ±/SEM of three independent experiments. Paired t-test: *P < 0.05. c MIF concentrations determined by ELISA in the supernatant of cells induced to differentiate for indicated time. Data are mean ±/SEM of indicated independent experiments (day 5: n = 6; day 7: n = 5; day 8: n = 3; day 10: n = 7). Paired t-test: *P < 0.05; **P < 0.001. d RT-qPCR analysis of MIF mRNA expression in four TET2-depleted (TET2 shRNA, gray bars) and control (SCR shRNA, black bars) human leukemic cell lines. Data are mean ±/SEM of three biological replicates. Unpaired t-test: *P < 0.05; **P < 0.001; ***P < 0.0001. e Immunoblot of SCR or TET2 shRNA infected leukemic cell lines sorted on GFP expression. Lower panels, quantification after actin normalization using Image J software. f MIF concentrations determined by ELISA in the supernatants of kasumi-1 (n = 5), M07e (n = 5), UT-7 (n = 4) and TF-1 (n = 5) cells transduced 24 h before with SCR (black squares) or TET2 (gray squares) shRNA. Data are mean ±/SEM of three biological replicates. Unpaired t-test: *P < 0.05; **P < 0.001; ***P < 0.0001. g Immunoblot of SCR or TET2 shRNA infected leukemic cell lines sorted on SCR expression. Lower panels, quantification after actin normalization using Image J software.

Significant difference between trTET2MUT and non-trTET2MUT CMML (Supplementary Fig. 2b). No difference in white blood cell, monocyte, neutrophil, lymphocyte count, and hemoglobin level was observed between the three groups whereas, interestingly, the platelet count was significantly lower in trTET2MUT patient subgroup (Supplementary Fig. 2c).

Immunoblot analysis of monocytes from 3 controls and 5 CMML patients (1 TET2WT, 4 trTET2MUT) further showed an increased expression of MIF protein in trTET2MUT patients (Supplementary Fig. 3a). MIF level was higher in the supernatant (18 h in serum-free medium) of trTET2MUT CMML-monocytes from 2 patients compared to healthy donors (Supplementary Fig. 3b, c) and in the supernatant of 3 trTET2MUT CMML patients at diagnosis compared to 3 healthy donor serum samples (Supplementary Fig. 3b, d). Finally, MIF concentrations in the supernatant of bone marrow aspirates from 10 healthy controls and 35 CMML patients (13 TET2WT, 19 trTET2MUT, and 3 non-trTET2MUT) (Supplementary Table 3) revealed a significant increase of MIF in trTET2MUT CMML samples (Fig. 2g). Together, these results identified a correlation between TET2 mutation, especially truncated mutants, and increased production of MIF.

**TET2 protein binds MIF promoter region.** Tet2 chromatin immunoprecipitation-sequencing (ChIP-seq) performed with mouse wild-type bone-marrow cells16 indicated the recruitment of Tet2 at the Mif promoter (chromosome 10, locus position 75,322,371–75,323,742 using the mouse genome NCBI Build 37/UCSC mm9). Using two distinct antibodies against Tet2 (Ab1: C2 and Ab2: sc-136926), we performed ChIP-qPCR to map Tet2 binding in 3 regions (R2–R4) spanning the MIF promoter (Fig. 3a) in control (shSCR) and TET2-depleted (shTET2) kasumi-1 cells. In control cells, Tet2 was enriched around the transcription start site (TSS) (Fig. 3b). In both humans and mice, downregulation of TET2 precluded chromatin immunoprecipitation, supporting the specificity of Tet2 binding. We then analyzed H3K4me3 and H3K27me3 histone marks and recruitment of active polyadenylase II (phosphorylated on serine 5, pSS Pol II) to the MIF promoter. H3K4me3 signal increased from the distal region of MIF promoter to the TSS region in shSCR cells, while it was decreased in shTET2 cells (Fig. 3c) as previously shown16. H3K27me3 was not detected at the MIF promoter (Fig. 3c). Finally, active polyadenylase II was recruited in the TSS area of the MIF gene at much higher levels in shTET2 than shSCR cells (Fig. 3d), in accordance with an increased transcription of MIF gene under TET2 depletion.

To determine the minimal DNA sequences regulating MIF promoter activity, we used fragments of the human MIF promoter, ranging from position −1083 to +129, into a luciferase reporter vector that was transiently expressed into SCR- and TET2-shRNA kasumi-1 cells. The −25/−129 and +44/+129 vectors drove only background luciferase activity while the other vectors induced a stronger luciferase activity in TET2-depleted compared to control cells (Fig. 4a). Since the −81/−129 sequence was sufficient to drive optimal MIF promoter activity, we explored the role of cis-acting regulatory elements in that region. Deletion of c-MYB, CREF, SP1F, AML1a, AP4, and HIF1 sites51 did not affect the luciferase activity measured in SCR- and TET2-shRNA kasumi-1 cells. Deletion of the SP1F and CREF sites decreased luciferase activity in both control and TET2-depleted cells, indicating that these sites are critical for MIF gene expression (Fig. 4b). Using the Transcription Element Search System (TESS, http://www.cbil.upenn.edu/tess/), we identified a potential EGR binding site overlapping the SP1F site (Fig. 4c). We noticed a higher recruitment of EGR1 to the R4 region of the promoter in CMML monocytes, including 11 TET2 WT and 10 TET2 MUT CMML samples (Fig. 2g). Next performed ChIP-qPCR in controls, trTET2MUT, and 3 non-trTET2MUT (Supplementary Table 3) revealed a significant increase of MIF in trTET2MUT CMML samples (Fig. 2g). Together, these results identified a correlation between TET2 mutation, especially truncated mutants, and increased production of MIF.

**TET2 interacts with EGR1.** In silico analysis using MethylPrimer Express (Applied Biosystems) identified a CpG island (CG ≥65% of 710 bp) within MIF gene promoter. Sequencing of bisulfite-treated DNA failed to identify any differential methylation of the minimal promoter sequence (−81/−129) in a cohort of 21 CMML monocytes, including 11 TET2WT and 10 TET2MUT samples, compared to 17 healthy donors, including 10 young and 7 age-matched donors, respectively (Supplementary Fig. 5). We noticed a higher recruitment of EGR1 to the R4 region of the MIF promoter in TET2-depleted cells, whereas EGR2 was only slightly more recruited and SP1 recruitment remained unchanged (Supplementary Fig. 4). Together, these results identify EGR1 recruitment to the minimal promoter sequence required for MIF gene expression.

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TET2-HA antibody, suggesting a stronger interaction with TET2 in the complex (Fig. 5d). Co-immunoprecipitation experiments in human blood monocytes confirmed TET2 interaction with EGR1 (Supplementary Fig. 6b, c) and validated the ability of TET2 to interact with HDAC1 and HDAC2 (Supplementary Fig. 6d).

**TET2 downregulation disrupts TET2/EGR1 interaction.** To explore if TET2 downregulation globally affects EGR1 recruitment to DNA in monocytes, we performed ChIP-seq in monocytes from two healthy donors and three TET2MUT CMML patients selected for having trTET2MUT with diverse variant allele frequencies (VAF). CMML1818 combined TET2 A1241fsX11
Fig. 2 Increased expression of MIF gene in TET2-mutant CMML monocytes. a-c Sequencing of total polyA-RNA was performed in sorted peripheral blood monocytes collected from age-matched healthy donors (n = 10, gray circles) and CMML patients (n = 60) including TET2WT (n = 17, blue squares) and TET2MUT (n = 43, orange triangles) cases. a MIF gene expression after count normalization. Data are mean +/- SEM of indicated biological samples. Mann–Whitney test using TET2WT as control, *P < 0.05. b Inverse correlation between TET2 and MIF gene expression in all samples (10 age-matched healthy donors and 60 CMML patients); R² = 0.2325, P < 0.0001. c RT-qPCR analysis (normalized to PPIA gene) of MIF mRNA expression in sorted peripheral blood monocytes of 19 young healthy donors (age < 65), 8 age-matched healthy donors (age > 64) and 146 CMML patients (TET2WT 56; TET2MUT 90). Data are mean +/- SEM of indicated biological samples. Dunnett’s multiple comparison test using Old HD as control, *P < 0.01, ns, non-significant. d Heatmap of the expression of a selection of cytokine and chemokine genes in the three cohorts. e TET2 and MIF gene expression after count normalization was compared in CMML monocytes of patients with truncating tr-TET2MUT (n = 34) and non-truncating (n = 9) non-tr-TET2MUT. Data are mean +/- SEM of indicated biological samples. Dunnett’s multiple comparison test using TET2WT (n = 17) as control, ****P < 0.0001, ns, non-significant. f MIF gene expression normalized to PPIA was compared in CMML monocytes of patients with truncating tr-TET2MUT (n = 68) and non-truncating (n = 22) non-tr-TET2MUT. Data are mean +/- SEM of indicated biological samples. Dunnett’s multiple comparison test using TET2WT (n = 56) as control, ***P < 0.001, ns, non-significant. g MIF concentrations in bone marrow fluid from healthy controls (N = 10), TET2WT (n = 12), tr-TET2MUT (n = 19) and non-tr-TET2MUT (n = 3) CMML patients. Data are mean +/- SEM of indicated biological samples. Dunnett’s multiple comparison test using Old HD as control, **P < 0.01, ns non-significant.

(VAF = 40%) and E1513Gf6x9 (VAF = 49%), CMML1900 exhibited a TET2 W564X mutant (VAF = 100%) and CMML1268 showed a TET2 H800fs variant (VAF = 16%). EGR1 recruitment was increased in CMML1818 and CMML1900 monocytes, both exhibiting high trTET2MUT allele frequencies, compared to CMML268 and control monocytes (Fig. 6a). Altogether, Cis-Regulatory Annotation System (CEAS) indicated that EGR1 was preferentially recruited to intergenic regions in control and CMML1268 monocytes, whereas it was highly recruited at TSS in the two CMML samples with high trTET2MUT allele frequencies (Fig. 6b). Ranking heatmaps centered on TSS confirmed EGR1 distribution around the TSS in the two CMML samples with high trTET2MUT VAF (Fig. 6c). Examples of EGR1 distribution around TSS are shown for RAD50 (Fig. 6d), RPL30, RPL37A, RPS13, RPS23, RPS3A, POLR1B, POLR1D, and POLR1E (Supplementary Fig. 7a). In control samples and CMML1268, EGR1 peaks were very similar and mainly intergenic, as illustrated for CEBPD (Fig. 6d), ALDH3B2 and BCL2 regions as well as intergenic areas on chromosome 2, 7, 9, 13, 16, and 22 (Supplementary Fig. 7b).

MACS2 algorithm identified 11,393 and 19,763 peaks in control samples, of which 2,069 (13%) were common to the two samples (Fig. 6e; Supplementary Data 2). The algorithm identified 17,324 and 32,651 peaks in CMML1900 and CMML1818 monocytes respectively, with 4,424 (18%) common peaks (Fig. 6f; Supplementary Data 3). Comparison of peak localization in monocytes from controls and CMML with trTET2MUT at high VAF identified only 29 common peaks (Fig. 6g; Supplementary Data 4), whereas comparison of peak localization in healthy donors and CMML1268 monocytes identified 1,510 common peaks (Fig. 6h; Supplementary Data 5), further arguing for CMML1268 being closer than other CMMls to TET2 wild-type monocytes regarding EGR1 peak localization (Fig. 6e). Gene Ontology (GO) analysis of EGR1-interacting genes, using peaks that are common to healthy donor and CMML monocytes, showed a dramatic change in CMML1900 and CMML1818, with a global enrichment in genes involved in RNA processing, ribosome biogenesis, and translation (Supplementary Fig. 8). Together, these experiments indicate that trTET2MUT mutants with decreased expression of TET2 gene induce chromatin remodeling that, at the level of MIF gene TSS, promotes the recruitment of EGR1 that may account for its overproduction.

MIF favors monocyte differentiation of myeloid progenitors. Tet2 deletion was associated with an increased monocyte count in ageing mice. We analyzed transcriptomic data generated from CD34+ cells sorted from healthy donor and CMML patients with and without TET2 mutation (Supplementary Table 4 and Supplementary Data 6). Interestingly, SPI1/PU.1 transcription factor expression, promoting monocytic differentiation, was increased, while the expression of GFI1, involved in granulocytic differentiation, was decreased in TET2MUT compared to TET2WT CD34+ cells (Fig. 7a). An inverse correlation was observed for the expression of these two genes in CD34+ samples (Fig. 7b). These observations could account for the increase monocytes to granulocytes ratio measured in the peripheral blood of TET2MUT CMML patients (Fig. 7c). Since mutations decreasing TET2 gene expression increased MIF production, and MIF could increase SPI1/PU.1 dependent transcriptional activity, we explored whether MIF affects myeloid cell differentiation. Cord blood CD34+ cells were cultured with SCF, FLT3L, IL-3, and G-CSF in the presence or absence of 20 ng/mL recombinant MIF for 48 h before bulk RNA-seq analysis: 33 genes were differentially expressed in the presence of MIF (Supplementary Fig. 9a). GO Molecular Function analysis of these differentially deregulated genes (DEGs) identified kinase activity (p value = 3.44e−6; FDR q value = 2.94e−3) (Supplementary Fig. 9b) and signaling receptor binding (p value = 3.34e−5; FDR q value = 1.43e−2) (Supplementary Fig. 9c) signatures. GO Biological Process analysis of these DEGs identified cell morphogenesis involved in differentiation (p value = 3.33e−6; FDR q value = 6.23e−3) (Supplementary Fig. 9d) and anatomical structure formation involved in morphogenesis (p value = 4.43e−5; FDR q value = 2.76e−2) (Supplementary Fig. 9e) signatures. Finally, CIBERSORT analysis identified more monocytes and macrophages in the culture when MIF was added (Supplementary Fig. 9f), further suggesting that MIF may promote monocytic differentiation of stem and progenitor cells. Cord blood CD34+ cells were cultured in medium with SCF, FLT3L, IL-3, and G-CSF in the presence or absence of recombinant MIF for 6 to 9 days before flow cytometry analysis. The addition of recombinant MIF significantly increased the fraction of CD14+ monocyte cells, at the expense of CD15+ granulocytes (Fig. 7d). Single cell analysis of cells collected at day 7 showed 7 clusters defined by the expression of characteristic genes (Supplementary Fig. 10a) and further validated by the ten most expressed genes (Supplementary Fig. 10b). MIF induced an increase in the number of cells in cluster 6 (monocytes) while this number was decreased in cluster 4 (granulocytes; Fig. 7e). Accordingly, MIF increased the expression in CSF1R gene, a SPI1/PU.1 transcription factor target, in cluster 6 while decreasing the expression of GFI1 gene in cluster 4 (Fig. 7f). These results further argue for the ability of MIF to promote the expansion of monocytes at the expense of granulocytes (Fig. 7g). Altogether, these data suggested that MIF overproduced by TET2-mutant cells could promote monocyte differentiation, generating a feedback loop that may promote disease progression.
In turn, the secreted cytokine favors the differentiation of CD34+ monocytes. Given the role of monocytes and MIF in atherosclerosis pathogenesis, MIF overproduction might also account for the cardiovascular risk identified in patients with TET2 mutated clonal hematopoiesis.

TET2 diversely modulates the expression of cytokine genes, according to the cell type and their environment. In immune cells such as dendritic cells and macrophages, as well as in microglia cells, Toll-like receptor (TLR) agonists upregulate TET2 expression. Conversely, TET2 binding to target genes, such as IL6, downregulates their expression in a catalytic activity-independent manner. TET2 favors inflammation resolution acting on immune cells and downregulating IL-6 production through HDAC1 and HDAC2 recruitment to IL6 gene promoter. TET2 also stimulates the inflammatory response of microglial cells. In a hypoxic tumor microenvironment, upregulated TET2 promotes IL6 gene expression in tumor cells, an effect that may involve the catalytic activity of dioxygenase since it is associated with the demethylation of IL6 promoter. Here, we show that, in the absence of inflammatory cue, TET2 downregulation in myeloid cells promotes the displacement HDAC1/HDAC2 from MIF promoter, leading to MIF gene expression. Strengthening this observation, HDAC inhibitors strongly decreased MIF expression in a variety of malignant cell lines and primary cells. Importantly, TET2 regulates MIF gene expression in resting monocytes while TET2-mediated IL6 regulation was detected at the recovery phase of macrophage activation. MIF is the main cytokine overproduced by resting monocytes in which TET2 expression is decreased, suggesting that overproduction of other cytokines by TET2-mutated cells may involve less direct effects of TET2 mutation.

Unlike TET1 and TET3, TET2 does not have a canonical CXXC domain binding unmethylated CpG, and IDAX/CXXC4 gene, leading to TET2 interaction with CpG islands, is not detectable in human monocytes (our data). Alternative candidates include the NF-kB inhibitor zeta (IkB) that targets Tet2 to Il6 gene proximal promoter in mouse myeloid cells exposed to lipopolysaccharides, the transcription factor forhead box O3 (Foxo3a) that interacts with Tet2 to promote the proliferation of mouse neural stem cells, and recently, the transcription factor EGR2, another member of EGR transcription factor family, that acts as an epigenetic pioneer to recruit TET2 to its binding sites in IL4/GM-CSF-differentiated dendritic cells from primary human monocytes.

Current evidence from animal models and clinical observations indicate that TET2 inactivation in hematopoietic stem cells may be an early event in the initiation of myeloid malignancies, and that additional hits are necessary for tumor progression. Inflammatory signals may provide these hits, as suggested in a

**Discussion**

The present study identifies a role for TET2 protein in the transcriptional regulation of MIF gene thanks to EGR1 transcription factor in monocytes. TET2 deficiency, e.g., as a consequence of truncating mutation, promotes MIF gene expression. In turn, the secreted cytokine favors the differentiation of CD34+ monocytes. Given the role of monocytes and MIF in atherosclerosis pathogenesis, MIF overproduction might also account for the cardiovascular risk identified in patients with TET2 mutated clonal hematopoiesis.

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Current evidence from animal models and clinical observations indicate that TET2 inactivation in hematopoietic stem cells may be an early event in the initiation of myeloid malignancies, and that additional hits are necessary for tumor progression. Inflammatory signals may provide these hits, as suggested in a
Fig. 4 Identification of a minimal proximal promoter of MIF gene. a Kasumi-1 cells stably transduced with SCR and TET2 shRNAs were transiently transfected with the pGL3 vector encoding the Luciferase gene alone (LUC) or LUC gene under control of indicated MIF promoter fragments. Cells were co-transfected with a Renilla luciferase construct for normalization and results are the ratio between luciferase and Renilla activities. Data are mean +/− SEM from at least six independent well experiments; Unpaired Students-t test, *P < 0.05.

b The same experiments were performed using the −157/+129 MIF promoter sequence in which mutations were induced in the DNA binding sites c-Myb, CREd, CREp, SP1d, SP1p, AML1a, AP4, and HIF1. Data are mean +/− SEM from seven independent well experiments; Unpaired t test, *P < 0.05.

c DNA consensus sequences for SP1d, SP1p, and EGR transcription factors in MIF proximal promoter.
mouse model of human chronic myelogenous leukemia in which IL-6 secreted by mature myeloid cells contributes to leukemic progenitor cell development\(^{59,60}\). Tet2 is upregulated by innate immune cells challenged with LPS and its knockdown delays inflammation resolution by precluding the repression of inflammatory cytokines as demonstrated for IL-6\(^{13,33}\). In the context of HIV-1 infection, TET2 protein degradation via Vpr protein similarly sustains IL-6 production, which enhances virus replication\(^{55}\). We noticed that, in the absence of additional infectious or inflammatory stimulus, MIF is the main cytokine overproduced by Tet2-deleted mice, as well as TET2 downregulated leukemic cells and TET2-mutated CML monocytes. Many parameters may influence the level of TET2 gene expression. In CML patients with a TET2 variant, truncation of the protein and the VAF of the mutated allele play essential roles. TET2 gene expression can also be decreased in the absence of gene mutation through poorly understood mechanisms. The multiple effects of MIF on immune cells have been largely investigated\(^{61}\). However, MIF effects on hematopoietic stem and progenitor cells remain poorly understood. In a mouse retroviral model of AML, MIF produced by a FLT3-mutated subclone was observed to favor the expansion of leukemia-
Here, we show that, when added to human healthy CD34+ cells in culture, MIF promotes their differentiation into monocytes, suggesting an autocrine/paracrine feedback loop in which MIF produced by mature clonal cells creates a microenvironment that promotes the development of a myeloproliferative syndrome.

MIF levels rise during infectious, inflammatory, and autoimmune diseases. MIF promotes carcinogenesis and plays a central role in atherosclerosis pathogenesis. In mouse models, Tet2 deletion accelerates atherosclerosis through enhanced secretion of cytokines, i.e., IL-8 and IL-1β, whereas Mif deletion reduces the aortic inflammatory response. The overexpression of MIF in Tet2-truncated monocytes, identified in the present study, could both promote the recruitment of additional inflammatory cells in atherosclerotic lesions and favor the monocyte production of IL-1β in response to inflammatory stimuli through its direct role in NLRP3 inflammasome activation. Of note, a G/C single-nucleotide polymorphism (rs755622) at position −173 of human MIF gene is associated to a higher susceptibility to develop coronary diseases and cancers. It would be worth determining whether this polymorphism impacts the outcome of Tet2-truncated CHIP, including the development of coronary diseases and overt myeloid malignancies.

Together, we have shown that Tet2-truncating mutations allow MIF gene expression through a catalytic domain-independent mechanism and provoke a chronic MIF overproduction by peripheral blood monocytes, in the absence of inflammatory signals. MIF could potentially promote the development of a myeloproliferative syndrome while it may accelerate atherosclerosis development. Anti-MIF antibodies were shown to exert protective effects in models of sepsis. Imalumab (BAX69), one of these humanized anti-MIF monoclonal antibodies, has completed phase I and II clinical testing with acceptable toxicity. As TET2 by itself is hardly druggable, targeting MIF should be considered as an alternative therapeutic strategy for preventing the development of atherosclerotic lesions and chronic myeloid malignancies in individuals with Tet2-truncating mutations.

**Fig. 6 EGR1 recruitment to DNA is modified by Tet2 mutation in monocytes.** Chip-seq experiments were performed using an anti-EGR1 antibody in sorted peripheral blood monocytes from 2 healthy donors (control 1 and 2) and three CMML patients (CMML1268: TET2 H800fs, VAF = 16%; CMML1818: TET2 A1241fsX1, VAF = 40% and TET2 E1513GfsX9, VAF = 49%; CMML1900: TET2 W564X, VAF = 100%).

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**Initiating cells** Here, we show that, when added to human healthy CD34+ cells in culture, MIF promotes their differentiation into monocytes, suggesting an autocrine/paracrine feedback loop in which MIF produced by mature clonal cells creates a microenvironment that promotes the development of a myeloproliferative syndrome.
Methods

In vitro granulo-monocyte differentiation. Umbilical cord blood samples were collected from healthy newborns with mother consent: AP-HP, Hôpital Saint-Louis, Unité de Thérapie Cellulaire, CRB-Banque de Sang de Cordon, Paris, France – N° d’autorisation: AC-2016-2759. Sorted CD34+ cells, using magnetic beads and AutoMacs system (Miltenyi Biotech), were depleted for TET2 and subjected to monocyte/macrophage differentiation as previously described48. Supernatants were collected at indicated day. For Fig. 7d, sorted CD34+ were cultured 24 h at 1 × 10^6 cells/mL in MEM-alpha medium (Thermo Fisher Scientific) with 10% heat inactivated fetal bovine serum (FBS), 1% penicillin/streptomycin and 2 mM L-Glutamine (Thermo Fisher Scientific), stem cell factor recombinant (SCF, 50 ng/mL; Immunex), interleukin-3 recombinant (IL-3, 10 ng/mL; Novartis), IL-6 recombinant (10 ng/mL; Peprotech), thrombopoietin recombinant (TPO, 10 ng/mL; Peprotech), Fms-like tyrosine kinase 3 recombinant (FLT-3, 50 ng/mL;
Fig. 7 MIF promotes monocyte differentiation. a CD34+ cells were sorted from the peripheral blood of healthy donor (n = 5) and CMML patients, without (n = 13) or with TET2 (n = 9) mutation, and analyzed by Agilent microarrays. Indicated gene expression was monitored (Log2 intensity). Data are mean +/- SEM of indicated biological samples. Dunnett’s multiple comparison test using healthy donor as control, P < 0.05, ns, non-significant. b Inverse correlation between SPI1 and GFI1 Log2 intensity expression in CD34+ samples. R² = 0.24, p = 0.02. c Monocytes to polymorphonuclear cell ratio in the peripheral blood of CMML patients without (n = 13) or with TET2 (n = 9) mutation. Mann-Whitney test: ** P < 0.005. d Cord blood CD34+ cells were induced to differentiate as described in Fig. 1, in the presence or the absence of 20 ng/mL MIF for 5–9 days before measuring the fraction of CD14+ cells by flow cytometry. Data are mean +/- SEM from eight independent experiments; Mann-Whitney test: ** P < 0.005. e Single cell analysis of cord blood CD34+ cells induced to differentiate as described in Fig. 1 with or without MIF at day 7. Umaps of cluster analysis at 17 dimensions with a resolution of 0.2. f Umaps of CSFIR and GFI1 gene expressions in monocyte and granulocyte clusters in CTRL and MIF conditions. Scales indicate the intensity of expression. g Percentage of monocytes and granulocytes in the myeloid compartment in CTRL and MIF conditions.
EGR1 knockdown in TET2-truncated mutated CMML monocytes. We used Stealth siRNAduplex (ThermoFisher Scientific) targeting EGR1 and Stealth RNAi® siRNA negative control (SCR) introduced into CMML monocytes by Lipofectamine 2000 (ThermoFisher Scientific). Briefly, 1 x 10^6 cells in 500 µL in a 24-well plate were transfected according to the manufacturer’s recommendations with Invivogen siRNAs targeting EGR1: s5EGR1-1: 5′-UUCCCGAGCCAAUUGGAAUUGCU-3′; s5EGR1-2: 5′-AGGAAAUUAAUGUCCUGGAGA-3′; s5EGR1-3: 5′-GAUCCUG/GACCGUGGAAUCCUU-3′. Knockdown efficiency was performed using RT-qPCR and MIF expression was addressed.

293 T transient transfection. 293T cells were transfected with pcDNA3-HA-TET2 and pcDNA3-EGR1 plasmids using Lipofectamine 2000 according to the manufacturer’s recommendation. 72 h after transfection, cells were harvested and lysed 20 min on ice in a buffer containing 50 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 10% Glycerol, 1 mM Na3VO4, and 1× Protease inhibitor cocktail (100 µL for 10 X 10^6 cells). Samples were then centrifuged 15 min at 4 °C at 18,000 × g and the supernatant containing the proteins was collected. 10 µg of anti-HA or anti-EGR1 antibody or negative control IgG were added and the samples incubated one night at 4 °C with agitation. 100 µL of Protein A/G PLUS-Agarose (Santa Cruz biotechnology) were incubated with the samples during 2 h at 4 °C with agitation. The complexes were then precipitated with 1 min centrifugation at 2000 × g. The supernatant was removed. The remaining beads were washed 5 times with 500 µL of lysis buffer without NP-40. 2× Laemmli with 0.1 M DTT and Protease inhibitor cocktail 1× was added on the beads and outputs. The samples were then boiled 10 min at 95 °C. Following SDS-PAGE and blotting, the membranes were incubated with either an anti-EGR1 antibody, an anti-HA, or an anti-HDAC1 antibody.

Immunoprecipitation experiment in primary monocytes. CD14+ cells were lysed 20 min on ice in a buffer containing 50 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 10% Glycerol, 1 mM Na3VO4 and 1× Protease inhibitor cocktail (100 µL for 10 x 10^6 cells). Samples were then centrifuged 15 min at 4 °C at 18,000 × g and the supernatant containing the proteins was collected. 10 µg of anti-HDAC1 (#95931, Active Motif) or an anti-HDAC2 (#95933, Active Motif) or negative control IgG were added for 10 x 10^6 cells and the samples incubated one night at 4 °C under agitation. One hundred µL of Protein A/G PLUS-Agarose (Santa Cruz Biotechnology) were incubated with the samples during 2 h at 4 °C under agitation. The beads were washed 5 times with 500 µL of lysis buffer without NP-40. 2X Laemmli with 0.1 M DTT and Protease inhibitor cocktail 1X was added on the beads and boiled 10 min at 95°C. Proteins were separated on polyacrylamide gel and transferred to nitrocellulose membrane (ThermoFisher Scientific). Membranes were blocked with 5% bovine serum albumin in PBS, with 0.1% Tween-20 (Sigma-Aldrich) for 40 min at RT, incubated overnight at 4 °C with anti-TET2 antibody (sc-16926, Santa Cruz biotechnology) (dilution 1/10000), washed in PBS-0.1% Tween-20, incubated further with HRP-conjugated secondary antibody (400 ng/mL) for 1 h at RT and washed again before analysis using Immunoblot Western Chemiluminescent HPR Substrate system (Millipore, Molsheim, France).

Gene expression microarray analysis. Gene expression in purified CD34+ cells was analyzed with Agilent® SurePrint G3 Human GE 8x60K Microarray (Agilent Technologies, AMADID-28004). After single color hybridization and array scanning, microarray images were analyzed using Feature Extraction software version (10.7.3.1) with default settings. Using LIMMA R package, data were normalized by the quantile method and analyzed (for single value of each transcript, the mean of each replicated probe was taken).

3 Single-cell RNAseq analysis. Raw BCL-files were demultiplexed and converted to Fastq format using bcl2fastq (version 2.20.0.422 from Illumina). Reads quality control was performed using fastqc (version 0.11.9) and assignment to the expected genome species evaluated with fastq-screen (version 0.14.0). Reads were pseudo-mapped to the Ensembl reference transcriptome v99 corresponding to the homosapiens GRCh38 build with kallisto (version 0.46.2) using its ‘bus’ subcommand and parameters corresponding to the 10X Chromium v3 scRNA-Seq v3 chemistry. The index was made with the kb-python (version 0.2.4) wrapper of kallisto. Barcode correction using whitelist provided by the manufacturer (10X Genomics) and gene-based reads quantification was performed with BUSTools (version 0.40.0).

Cell barcode by symbol count table was loaded in R (version 4.0.4) using the BiocMultiSubpackage (version 1.5.3). To call real cells from empty droplets, we used the emptyDrops function from the dropletUmi package (version 1.10.3), which assesses whether the RNA content associated with a cell barcode is significantly distinct from the ambient background RNA present within each sample. Barcodes with p-value < 0.001 (Benjamini–Hochberg-corrected) were considered as legitimate cells for further analysis.

The count matrix was filtered to exclude genes detected in less than five cells, cells with less than 1500 UMIs or less than 200 detected genes, as well as cells with mitochondrial transcripts proportion higher than 20%. Cell cycle scoring of each cell was performed using two methods: the CellcycleScoring function from the Seurat package (version 4.0.0) and the cycle function from Scan (version 1.18.5). Barcodes corresponding to doublets were identified and discarded using the union of two methods: scdbFinder (version 1.4.0) using default parameters, and scds (version 1.6.0) with its hybrid method using default parameters. We manually verified that the cells identified as doublets did not systematically correspond to cells in G2M phase.

Seurat (version 4.0.0) was applied for further data processing. The SCTransform normalization method was used to normalize, scale, select 3000 Highly Variable Genes and regress out bias factors (the number of detected transcripts and the proportion of ribosomal transcripts). The number of PCA dimensions to keep for further analysis was evaluated by assessing a range of reduced PCA spaces using 3 to 49 dimensions, with a step of 2. For each generated PCA space, Louvain clustering of cells was performed using a range of values for the resolution parameter from 0.1 to 1.2 with a step of 0.1. The optimal space was manually evaluated as the one combination of kept dimensions and clustering resolution resulting in the best structure (clusters homogeneity and compacity) in a Uniform Manifold Approximation and Projection space (UMAP). For the 2 samples, 17 dimensions were retained with a resolution of 0.2.

Marker genes for Louvain clusters were identified through a «one versus others» differential analysis using the Wilcoxon test through the FindAllMarkers function from Seurat, considering only genes with a minimum log fold-change of 0.5 in at least 75% of cells from one of the groups compared, and FDR-adjusted p-values < 0.05 (Benjamini–Hochberg method). Gene visualization was done using Cerebro (version 1.2.2).

Statistics and reproducibility. All statistical analyses (Paired and unpaired t-tests, Mann–Whitney test, Dunnett’s multiple comparison tests, correlation using linear regression) were performed using Graph-Pad Prism software (GraphPad Software, La Jolla, CA, USA). Values of p < 0.05 were considered statistically significant. The used statistical test, the p value, and the number of samples are indicated in the respective Figure legends.

Data availability
Control and CMML monocyte RNA-seq data have been deposited in the NCBI Gene Expression Omnibus database under accession number GSE165305 and GSE186824. Chip-seq datasets are available in the Array Express database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB_6305 and E-MTAB_11132. Cord blood seq datasets are available in the Array Express database at EMBL-EBI (www.ebi.ac.uk/). The source data underlying all graphs and charts are provided as Supplementary Data 7.

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References
1. Ito, S. et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxycytosine. Science 333, 1300–1303 (2011).
2. Lu, F., Liu, Y., Jiang, L., Yamaguchi, S. & Zhang, Y. Role of Tet proteins in enhancer activity and telomere elongation. Genes Dev. 28, 2103–2119 (2014).
3. Wu, X. & Zhang, Y. TET-mediated active DNA demethylation: Mechanism, function and beyond. Nat. Rev. Genet. 18, 517–534 (2017).
4. Costa, Y. et al. NANG-dependent function of TET1 and TET2 in establishment of pluripotency. Nature 495, 370–374 (2013).
35. Sano, S. et al. Tet2-mediated clonal hematopoiesis accelerates heart failure through a mechanism involving the IL-1β/NLRP3 inflammasome. *J. Am. Coll. Cardiol.* 71, 875–886 (2018).

36. David, J. R. Delayed hypersensitivity in vitro: Its mediation by cell-free substances formed by lymphoid cell-antigen interaction. *Proc. Natl Acad. Sci. USA* 56, 72–77 (1966).

37. Bloom, B. R. & Bennett, B. Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. *Science* 153, 80–82 (1966).

38. Calandra, T. & Roger, T. Macrophage migration inhibitory factor: A regulator of innate immunity. *Nat. Rev. Immunol.* 3, 791–800 (2003).

39. Calandra, T. et al. MIF and ghrelin-induced modulator of cytokine production. *Nature* 377, 68–71 (1995).

40. Calandra, T. et al. Protection from septic shock by neutralization of macrophage migration inhibitory factor. *Nat. Med.* 6, 164–170 (2000).

41. Bernhagen, J. et al. MIF is a noncoagulant ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat. Med.* 13, 587–596 (2007).

42. Tilstam, P. V., Qi, D., Leng, L., Young, I. & Bucala, R. MIF family cytokines in cardiovascular diseases and prospects for precision-based therapies. *Expert Opin. Ther. Targets* 21, 671–683 (2017).

43. Kang, I. & Bucala, R. The immunobiology of MIF: Function, genetics, and prospects for precision medicine. *Nat. Rev. Rheumatol.* 15, 427–437 (2019).

44. Burgers, J. et al. Reduction of the aortic inflammatory response in spontaneous atherosclerosis by blockade of macrophage migration inhibitory factor (MIF). *Atherosclerosis* 184, 28–38 (2006).

45. Bozza, M. et al. Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis. *J. Exp. Med.* 189, 341–346 (1999).

46. Jaiswal, S. et al. Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p38. Regulatory role in the innate immune response. *Proc. Natl Acad. Sci. USA* 99, 345–350 (2002).

47. Shin, M. S. et al. Macrophage migration inhibitory factor regulates U1 small nuclear RNP immune complex-mediated activation of the NLRP3 inflammasome. *Arthritis Rheumatol.* 71, 109–120 (2019).

48. Priori, E. et al. Inhibition of TET2-mediated conversion of 5-methylcytosine to 5-hydroxymethylcytosine disturbs cytidine and guaninonucleoside differentiation of human hematopoietic progenitors. *Blood* 118, 2551–2555 (2011).

49. Izzyra, R. et al. Clonal architecture of chronic myelomonocytic leukemias. *Blood* 121, 2186–2198 (2013).

50. Meirevede, J. et al. Mutation allele burden remains unchanged in chronic myelomonocytic leukemia responding to hypomethylating agents. *Nat. Commun.* 7, 10767 (2016).

51. Roger, T., Ding, X., Chanson, A. L., Renner, P. & Calandra, T. Regulation of constitutive and microbial pathogen-induced human macrophage migration inhibitory factor (MIF) gene expression. *Eur. J. Immunol.* 37, 3509–3521 (2007).

52. Roger, T., David, J., Glauser, M. P. & Calandra, T. MIF regulates innate immune responses through modulation of Toll-like receptor 4. *Nature* 414, 920–924 (2001).

53. Lv, L. et al. Vpr targets TET2 for degradation by CRL4 VprBP E3 ligase to sustain IL-6 expression and enhance HIV-1 replication. *Mol. Cell* 70, 961–970.e5 (2018).

54. Carrillo-Jimenez, A. et al. TET2 regulates the neuroinflammatory response in microglia. *Cell Rep.* 29, 697–713.e8 (2019).

55. Ihoh, H. et al. TET2-dependent IL-6 induction mediated by the tumor microenvironment promotes tumor metastasis in osteosarcoma. *Oncogene* 37, 2903–2920 (2018).

56. Lugrin, J. et al. Histone deacetylase inhibitors repress macrophage migration inhibitory factor (MIF) expression by targeting MIF gene transcription through a local chromatin deacetylation. *Biochim. Biophys. Acta - Mol. Cell Res.* 1793, 1749–1758 (2009).

57. Ko, M. et al. Modulation of TET2 expression and 5-methylcytosine oxidation by the CXCR4 domain protein IDAX. *Nature* 497, 122–126 (2013).

58. Li, X. et al. Ten-eleven translocation 2 interacts with forkhead box O3 and regulates adult neurogenesis. *Nat. Commun.* 8, 15903 (2017).

59. Reynaud, D. et al. IL-6 controls leukemic multipotent progenitor cell fate and contributes to chronic myelogenous leukemia development. *Cancer Cell* 20, 561–573 (2011).

60. Welner, R. S. et al. Treatment of chronic myelogenous leukemia by blocking macrophage migration inhibitory factor (MIF). *Nat. Med.* 14, 158–164 (2008).

61. Fuster, J. J. et al. Clonal hematopoiesis associated with TET2 de...
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Competing interests

The authors declare no competing interests.

Additional information

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