Review

Dissecting TET2 Regulatory Networks in Blood Differentiation and Cancer

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Simple Summary: Bone marrow disorders such as leukemia and myelodysplastic syndromes are characterized by abnormal healthy blood cells production and function. Uncontrolled growth and impaired differentiation of white blood cells hinder the correct development of healthy cells in the bone marrow. One of the most frequent alterations that appear to initiate this deregulation and persist in leukemia patients are mutations in epigenetic regulators such as TET2. This review summarizes the latest molecular findings regarding TET2 functions in hematopoietic cells and their potential implications in blood cancer origin and evolution. Our goal was to encompass and interlink up-to-date discoveries of the convoluted TET2 functional network to provide a more precise overview of the leukemic burden of this protein.

Abstract: Cytosine methylation (5mC) of CpG is the major epigenetic modification of mammalian DNA, playing essential roles during development and cancer. Although DNA methylation is generally associated with transcriptional repression, its role in gene regulation during cell fate decisions remains poorly understood. DNA demethylation can be either passive or active when initiated by TET dioxygenases. During active demethylation, transcription factors (TFs) recruit TET enzymes (TET1, 2, and 3) to specific gene regulatory regions to first catalyze the oxidation of 5mC to 5-hydroxymethylcytosine (5hmC) and subsequently to higher oxidizedcytosine derivatives. Only TET2 is frequently mutated in the hematopoietic system from the three TET family members. These mutations initially lead to the hematopoietic stem cells (HSCs) compartment expansion, eventually evolving to give rise to a wide range of blood malignancies. This review focuses on recent advances in characterizing the main TET2-mediated molecular mechanisms that activate aberrant transcriptional programs in blood cancer onset and development. In addition, we discuss some of the key outstanding questions in the field.

Keywords: DNA methylation; TET2; chromatin; transcription factors; gene regulation; blood malignancies

1. Introduction

CpG methylation is the most common DNA modification found in the mammalian genome [1], playing an essential role in development and cancer [2,3]. DNA demethylation can be either passive, by dilution of DNA methylation after each cell division, or active when initiated by Tet dioxygenases [4]. During active demethylation, the Ten-eleven-translocation (TET) family of enzymes first catalyze the iterative oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and subsequently to higher oxidized derivatives, 5-formylcytosine (5fC) and 5-carboxycytosine (5CaC) [5–7]. These higher oxidized forms of cytosine, in turn, can either be lost during replication or enzymatically removed, restoring unmodified cytosine and alleviating transcriptional repression typically associated with 5mC residues [4]. However, 5hmC and higher oxidized derivatives should be considered not only as mere transient states in the DNA demethylation path but as bona fide epigenetic marks as illustrated by their complex network of specialized readers [8].
In the context of the hematopoietic system, TET family members have been involved in naturally occurring and experimentally induced cell fate decisions [9–14]. Among the TETs, TET2 is the most broadly expressed and frequently mutated gene in blood malignancies [15–20]. TET2 mutational profiles show inactivating mutations occurring along the whole gene coding region and are not only restricted at the 3′ region where the catalytic domain is located [15,17,21]. Therefore, TET2 epigenetic regulation during blood differentiation might be mediated not only by its catalytic activity but also by its association with critical partners [22,23].

Here we review the current understanding of TET2 functions in normal and malignant hematopoiesis, providing an extensive overview of the intricate molecular mechanisms controlling gene expression, protein stability and function, and enzyme’s genome recruitment.

2. Mechanisms of TET2 Protein/Enzymatic Regulation

Several mechanisms regulating TET2 expression and activity have been elucidated in the last decade. Here we summarize main control systems, encompassing basal post/transcriptional regulation, direct protein modulation through post-translation modifications (PTMs), and enzymatic substrate availability.

2.1. Transcriptional Regulation

Some transcriptional factors have been defined as direct regulators of TETs’ gene expression. In mouse embryonic stem cells (ESCs), Tet1 and Tet2 are positively regulated by the pluripotency TF Oct4 that binds to conserved non-coding sequences in both genes [24]. Accordingly, upon ESC differentiation, Tet1 and Tet2 levels decrease due to Oct4 depletion [24].

A similar regulatory mechanism was described for the CXXC-DNA binding domain protein Rinf (CXXC5), whose depletion leads to decreased Tet1 and Tet2 expression [25]. During myeloid cell fate commitment, Tet2 expression is boosted by the action of the myeloid transcription factor CEBPα, which binds to Tet2 enhancer regions [26]. CEBPα might exert its transcriptional control by recruiting Tet2 protein itself to Tet2 gene’s distal regulatory regions leading to their demethylation and activation [10]. Of note, a mutant form of CEBPα (Brm2), recapitulating naturally occurring mutations in AML patients [27], failed to demethylate the Tet2 enhancers [10]. In addition, histone deacetylase 4 (HDAC4) protein has been recently described as a positive regulator of TET2 expression in the context of MDS and AML [28]. Finally, in regulatory T cells (Treg), Tet1 and Tet2 are regulated in response to hydrogen sulfide (H2S) through the action of the sulfhydrating nuclear transcription factor Y subunit beta (NFYB) [29].

2.2. Post-Transcriptional Regulation

miRNAs regulatory networks targeting TET2 mRNAs have been proposed as the primary post-transcriptionally regulatory mechanism during blood differentiation and in myeloid malignancies. High-throughput screens identified a large subset of TET2 3’UTR targeting miRNAs with different efficiencies. Induced expression of those led to an array of leukemic traits such as myeloid lineage bias, phenocopying to an extent, a direct TET2 loss [30]. In a related study, miR-22 (also targeting TET2 mRNA) was detected overexpressed in MDS patients. Mechanistically, miR-22 overexpression leads to reduced genome-wide levels of 5hmC, increased self-renewal, and myeloid skewing [31]. Also, in MDS, miR-9 and miR-34a indirectly control TET2 by post-transcriptionally regulating SIRT1 levels, which affect the TET2 protein function at a post-translational level (See Section 2.3) [32].

2.3. Post-Translational Regulation

Although TET2 protein levels are mainly regulated via transcriptional mechanisms, TET2 post-translational modifications might be involved in rapidly fine-tuning protein levels in response to external cues.
During ESC differentiation, the CXXC-DNA binding domain protein IDAX (CXXC4) recruits TET2 to DNA, activating caspases that cleave the TET2-IDAX complex leading to TET2 protein depletion [33]. Similarly, TET proteins have been described as direct substrates of the calpain family of proteases [34]. Calpain1 regulates the degradation of Tet1 and Tet2 in mouse pluripotent ESCs and calpain2 of Tet3 during ESCs neural differentiation. These negative regulatory mechanisms ensure correct global 5hmC level maintenance and expression of lineage-specific genes in ESCs [34].

In addition, TET2 protein can be largely post-translationally modified (PTM) by (de)phosphorylation, (de)acetylation, O-GlcNAcylation, or ubiquitylation, among others [32,35–38] (Figure 1). The specific output driven by particular PTMs on TET2 activity is cell type and amino acid residue-specific. For instance, cytokine receptor-associated JAK2 (in response to FLT3 or EPO/SCF signaling in blood progenitor cells) phosphorylates TET2 at tyrosine residues 1939 and 1964, leading to enhanced enzymatic activity [35] (Figure 1). However, TET2 tyrosine residues 1939 and 1964 are not the only phosphorylatable residues since the whole N-terminus region of the protein (constituting a large disordered domain) is usually highly phosphorylated in ESCs [36] (Figure 1). Interestingly, the O-linked N-acetylgalactosaminyltransferase (OGT), a strong TET interactor [36,39–42], adds O-GlcNAcylation groups to serine and threonine residues of TET2, thereby reducing the number of available phosphorylation sites and their site occupancy [36]. Once again, the described phosphorylation vs. O-GlcNAcylation mechanism highlights the fine-tuning TET proteins might undergo for correct localization and activity according to external signals [36].

Figure 1. Schematic representation of TET2 post-translational modifications (PTMs) and most frequently found TET2 mutations. In the upper half, compiled PTM data based on mass spectrometry (MS) and in silico predictions [35,36,38,43,44]. In grey, proposed modifications with an undefined function. In color, fully characterized PTMs and their impact on TET2 activity. In the bottom half, a compilation of TET2’s most frequent mutations and their type (truncating, missense, or splice mutations). Filtering was done from combined 12,845 samples from 35 studies where the mutation was found in >5 different patients. Data from ‘Myeloid’ dataset from cbioPortal (https://www.cbioportal.org/, accessed on 19 January 2022) [45,46].

TET2 activity can also be regulated through protein (de)acetylases. This is the case of the NAD-dependent deacetylase sirtuin-1 (SIRT1) that removes acetylation at TET2 specific lysine residues K1472, K1473, and K1478, increasing protein’s enzymatic activity [32].
Consequently, reduced SIRT1 activity in human HSPCs leads to the onset of an MDS-like disease recapitulating the phenotype observed in TET2-mutated MDS patients [32]. Whereas TET2 global deacetylation mediated by histone deacetylases, 1 and 2 (HDAC1 and 2) leads to reduced enzymatic activity triggering the emergence of abnormal DNA methylation profiles typically associated with cancer [38]. Zhang and co-workers also studied the effects on TET2 stability/activity mediated by histone acetyltransferase p300 (enzymatic counterpart of the HDAC1/2 enzymes). p300 was shown to acetylate the TET2 N-terminus region leading to increased protein activity, stability, and partnering with other proteins such as DNMT1 [38]. TET2/DNMT1 complex might prevent abnormal promoter methylation typically observed upon exposure to oxidative stress [38].

Finally, ubiquitylation of TET2 has been described to regulate its chromatin association [43]. CRL4 (VprBP) E3 ligase, a member of the ubiquitin ligase complex, has been shown to interact with the cysteine-rich domain of TET2 and promote K1299 monoubiquitylation, enhancing its chromatin association [43]. On the contrary, USP15-dependent K1299 deubiquitylation leads to decreased TET2 activity [44].

2.4. Enzymatic Regulation

Regarding catalytic activity, TET enzymes are Fe2+/α-KG-dependent dioxygenases. Metabolite and cofactor availability constitute another relevant layer of protein regulation potentially influencing hematopoiesis and leukemic development. Interestingly, experimentally-induced ascorbate (VitC) depletion leads to increased HSC function and compartment expansion, resembling the aberrant self-renewal phenotype typically observed upon Tet2 depletion in HSCs [47–49]. In addition, Cimmino and co-workers elegantly demonstrated the potential of VitC treatment to rescue an aberrant self-renewal phenotype initiated upon Tet2 in vivo depletion [50]. On the contrary, 2-Hydroxyglutarate (2-HG), an oncometabolite produced in IDH1/2 mutated patient cells, competitively inhibits TET2 catalytic activity [50,51], resulting in genome-wide DNA hypermethylation and impaired myeloid differentiation [52]. Similarly, mutations in other metabolic players such as fumarate hydratase (FH) and succinate dehydrogenase (SDH) lead to fumarate and succinate accumulation that inhibit Tet enzymatic activity even with stable α-KG levels [53]. The interplay between these metabolic intermediaries and TET2 might also be directly relevant in clinics as mutations in iron and 2-oxoglutarate-binding sites have been reported in AML patients [15,54]. Finally, although essential for TET catalytic activity, oxygen has been described as a minor direct regulator of TET function in physiological settings [55]. However, low oxygen levels might indirectly regulate TET2 expression and activity in leukemic cells through a mechanism involving the activation of the hypoxia-inducible factor 1α (HIF1α) [56].

3. Partner-Instructed Tet2 Genomic Recruitment during Development and Cancer

Regulation of TET activity by controlling enzymes’ genomic distribution allows surgically modifying DNA methylation at particular genomic loci and only in specific cell types. Since TET2 lacks the low-affinity (CXXC) DNA binding domain, which is present in TET1 and TET3 [33], the enzyme must always be recruited to specific genomic locations through a ‘partner’ protein such as transcription factors.

Here we present an elaborated list of potential Tet2 interactors/recruiters playing relevant roles in different biological settings (Figure 2).
Figure 2. Graphical overview of TET2 protein partners/interactors. In the center, the human TET2 AlphaFold Database predicted protein structure (last updated on 1 July 2021 with AlphaFold v2.0) [57,58]. Encompassing the TET2 protein structure, color-coded bubbles cluster TET2 interactors based on previously described cell fate implications and physiological responses when cooperating with TET2. GR: glucocorticoid receptor.

3.1. During Embryonic Stem Cell Fate Decisions

Tet2 is considered an important pluripotency regulator playing critical roles during experimentally-induced pluripotency establishment [10,59–64] and in pluripotency maintenance [65–67]. In addition, Tet enzymes are essential for proper pluripotency exit during embryo development [68,69] and in ES cell differentiation [70,71].

Molecular mechanisms underlying the Tet2 pluripotency-related functions have recently been partially uncovered by systematically identifying and characterizing critical Tet2 interactors in ESCs. The naïve pluripotency TF Nanog was the first pluripotency-related protein identified interacting with Tet2 and Tet1 [62]. Thus, explaining Tet2 and Tet1 bound at regulatory regions of pluripotency genes in ESCs [62,66]. However, Tet2 is also functionally associated with other pluripotency-related TF at these regions, including Sall4 [72] and Prdm14 [73]. Sall4 acts in concert with Tet1 to firstly oxidize 5mC CpG residues into 5hmC and later with Tet2 to further oxidize them into 5fC and 5caC residues. However, no direct physical interaction between Sall4 and Tet2 was observed in ESCs [72].
Thus, suggesting the involvement of additional factors in this functional interplay. On the contrary, Prdm14, through direct association with Tet2, drives active demethylation at pluripotency and germline-associated genes such as Tcl1, Tcfap2c, and Spo11, Syce3, respectively [73].

Additionally, Tet2 has been described to interact with a handful of non-canonical pluripotency TFs equally playing essential roles in regulating its functions in ESCs. The latter includes the CXXC-DNA binding domain proteins Idax (CXXC4) and Rinf (CXXC5), potentially influencing Tet2 functions in differentiation [33] and pluripotency [25], respectively. Rinf co-occupies with Tet2 and Nanog distal regulatory regions of relevant pluripotency genes (such as Oct3/4, Sox2, and Nanog itself), positively regulating their transcription. The RNA-binding protein PSPC1 associates with Tet2 and targets the enzyme to the MERVL endogenous retroviral elements in ESCs [67]. Of note, these retroviral elements have been described to regulate the expression of 2C-genes in ESCs [74,75]. Once recruited to MERVL elements, Tet2 contributes to their transcriptional and epitranscriptomic regulation by recruiting HDACs to chromatin and oxidizing MERVL transcripts, respectively [67].

As mentioned above, Tet2 is also required during experimentally induced pluripotency establishment. To uncover factors associated with Tet2 in this biological setting, we analyzed DNA (hydroxy)methylation dynamics during rapid and highly efficient iPSC reprogramming systems [59,60,76]. Here, Tet2 is recruited by the Klf4 and Tfcp2l1 transcription factors to drive active enhancer demethylation of chromatin and pluripotency-related genes to reprogram cell fate [10].

Of note, a prominent study has recently identified common molecular mechanisms controlling DNA methylation during embryo development and in the leukemic transformation [77]. Thus, highlighting the potential health implications of studying Tet-mediated DNA demethylation in embryonic stem cells.

3.2. During Blood Cell Fate Decisions

Tet2 role in hematopoiesis and its influence on the DNA methylome has also been widely explored in the context of physiological and pathological conditions. Several studies have described how Tet2 protein deficiency can lead to aberrant cell fate decisions by extensively altering the DNA methylome during hematopoiesis. Here we recapitulate the role of Tet2 recruitment to specific genomic regions in different hematopoietic developmental pathways:

3.2.1. During Myeloid Cell Fate Decisions

Tet2-mediated epigenetic gene regulation is crucial during myeloid cell development. C/EBPα is an essential factor in the differentiation process from HSPCs to GMPs [78]. C/EBPα alone, through its pioneer activity, or in concert with PU.1, binds regulatory regions of myeloid genes to establish the myeloid cell fate [79]. To this end, C/EBPα directly activates Tet2 expression [26] and, through direct interaction with the enzyme, targets it to regulatory regions of myeloid genes such as Klf4 or Chd7, driving their active DNA demethylation and subsequent enhancer activation [10]. RUNX1, another key hematopoietic transcription factor, has also been described to interact with TET2 [80]. Thus, it potentially leads to the DNA demethylation observed at RUNX1 binding sites (including promoters of PTPN22, RUNX1, and RUNX3, among others) during hematopoietic differentiation [80]. Wilm’s tumor (WT1) gene encodes a sequence-specific transcription factor found mutated in a mutually exclusive manner with TET2 in AML patients [20]. Interestingly, WT1 directly associates with TET2 [81] to regulate WT1-target genes (including Wnt and MAPK signaling-related genes such as BTRC, DACT1, and TBL1X), preventing AML onset [81,82].

However, TET2 activity is not only relevant during early myeloid commitment but also in myeloid terminal differentiation [83]. In this regard, during monocyte-to-osteoclast differentiation, the master myeloid TF PU.1 recruits TET2 to promoters of key osteoclast-
genes (including ACP5, CTSK, and TM7SF4), leading to their demethylation and cell fate transition [84]. Tolerogenic dendritic cells (tolDCs) are also terminally differentiated myeloid cells with potent immunosuppressive properties. Mechanistically, the tolerogenic phenotype is acquired through a synergistic interplay between the glucocorticoid receptor (GR) and the specific myeloid transcription factor MAFB. Both TFs target TET2 at genomic loci exclusively demethylated in tolDCs [85]. In addition, EGR2, an essential transcription factor during IL4/GM-CSF-driven monocyte (MO) to monocyte-derived DCs (moDCs) differentiation, has also been described to interact with TET2 and initiate DNA demethylation at both EGR2 stable and transient binding sites [86]. However, TET2 genomic recruitment is not always associated with positive regulation of gene expression in dendritic cells. For instance, Zhang and co-workers identified that IκBζ-dependent Tet2 targeting the Il6 locus leads to Hdac2 recruitment. Thus, finally leading to Il6 gene repression and inflammation resolution [87].

3.2.2. Erythroid Lineage

HSPC commitment towards erythroid lineage also correlates with 5hmC accumulation and increased expression of erythroid-specific genes such as EPOR, GATA1, and HBB [88]. 5hmC accumulation might be mediated by Klf1-dependent Tet2 recruitment at erythroid-specific genes. Of note, both factors have been recently described to interact upon Jak2-driven Tet2 phosphorylation [35].

3.2.3. B-Cell Lineage

B cell differentiation is tightly regulated at the epigenetic level. B cell maturation is characterized by extensive reshaping of the cellular methylome [89]. Tet2 might contribute to the process by interacting with B-cell master regulators such as EBF1 [90], IRF4/8, E2A, and PU.1 or BATF and driving focal demethylation at regulatory regions of key B-cell loci (including IgK or Aicda) [91–93].

3.2.4. T-Cell Lineage

5hmC is accumulated at genes encoding key regulators of T cell identity, development, and differentiation [94]. However, what factors recruit Tet enzymes to the T-cell key regulatory regions is poorly known. In regulatory T cells (Tregs), Tet1 and Tet2 are upregulated in response to the sulfhydrating nuclear transcription factor Y subunit beta (NFYB) [29]. Tet1 and Tet2 are targeted by the activated forms of Smad3 and Stat5 to the Foxp3 promoter favoring its hypomethylation and stable gene expression [29].

3.3. In Response to External Stimuli

Tet2 has also been implicated in genome stability. Upon exposure to oxidative stress (OS), protein complexes containing DNMTs and TET2 are recruited to the specific genomic regions to repair the damaged DNA properly. TET2 depletion leads to reduced 5hmC levels at the damaged regions and impaired repair [95–98]. As a result of these exciting findings, several epigenetic regulatory mechanisms that tie Tet2 to protection against abnormal DNA methylation during stress have been proposed. For instance, Zhang and co-workers showed that upon OS, TET2 interacts with the thymidine glycosylase (TDG), an enzyme involved in the DNA active demethylation pathway. Then, both proteins are recruited to chromatin in a DNMT1 dependent manner, a process that is enhanced when TET2 is acetylated [38]. Therefore, the latter mechanism is suggested to protect against the acquisition of abnormal DNA methylation at typically unmethylated gene regulatory regions.

Another Tet2 interactor in the context of DNA damage response is the SMAD nuclear interacting protein 1 (SNIP1) that regulates the expression of relevant c-MYC target genes involved in apoptosis. Therefore, reduced SNIP1 levels lead to diminished 5hmC levels and gene expression at c-MYC target genes [99].
Finally, chemotherapeutic drugs have also been described to trigger hydroxymethylation changes in a mechanism mediated by the interaction between the promyelocytic leukemia protein (PML) and TET2, linking the protein to direct clinical applications [100].

4. TET2 Loss of Function in Blood Malignancies

DNA methylation aberrations are considered hallmarks of cancer onset and progression [101–103]. TET2 loss of function mutations are frequently found in patients suffering from myeloid malignancies such as acute myeloid leukemia, myeloproliferative neoplasms, or myelodysplastic syndromes [15,20,104] (Table 1). However, these mutations are also commonly observed in other blood malignancies, including B and T lymphomas [105–109], such as the angioimmunoblastic T-cell lymphoma (AITL), showing the highest incidence of TET2 mutations (roughly 80%) among all blood cancer types (Table 1). The broad TET2 mutational profile observed in the hematopoietic system has awakened great interest in the field to unravel the molecular mechanisms underlying TET2 involvement in the onset of preleukemic and leukemic diseases.

Table 1. List of TET2 mutation frequencies in hematologic malignancies and their prognostic value. Combined 12845 samples from 35 studies were analyzed according to cancer type and detailed classification based on WHO Classification of Hematologic Malignancies or The French–American–British (FAB) classification. Data from ‘Lymphoid’ and ‘Myeloid’ datasets from cbioPortal (https://www.cbioportal.org/, accessed on 19 January 2022) [45,46] and selected referenced studies [108,110–112]. AML = Acute myeloid leukemia, AMML = Acute Myelomonocytic Leukemia, AML-M5 = Acute Monoblastic/Monocytic Leukemia, CML = Chronic Myelogenous Leukemia, MPN = Myeloproliferative Neoplasms, MDS = Myelodysplastic Syndromes, CMML = Chronic Myelomonocytic Leukemia, CLL/SLL = Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma, DLBCL = Diffuse Large B-Cell Lymphoma, AITL = Angioimmunoblastic T-cell lymphoma, NOS = Not Otherwise Specified, N/A = Not Available.

| Cancer Type                   | Detailed Cancer Type                                      | Frequency (%) | Prognosis          |
|-------------------------------|----------------------------------------------------------|---------------|--------------------|
| AML (399/4014) 10%            | AML (unspecified)                                        | 9             | Unfavorable [113]  |
|                               | AML, NOS                                                 | 17            | Unfavorable [113]  |
|                               | AML with Bi-allelic Mutations of CEBPA                   | 24            | Unfavorable [114]  |
|                               | AML with t(3)(q21.3;q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM | 8             | N/A                |
|                               | AML with mutated NPM1                                    | 18            | Unfavorable [19]   |
|                               | AML with Myelodysplasia Related Changes                  | 8             | Unaffected [115]   |
|                               | AML with Recurrent Genetic Abnormalities                  | 5             | N/A                |
|                               | AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1               | 13            | N/A                |
|                               | AMML                                                     | 8             | N/A                |
|                               | AML-M5                                                   | 15            | N/A                |
| MDS/MPN (1023/2700) 38%       | MDS (unspecified)                                        | 25            | Favorable [116]    |
|                               | MDS, Unclassifiable                                     | 13            | Favorable [116]    |
|                               | MDS with Excess Blasts (unspecified)                     | 22            | Favorable [116]    |
|                               | MDS with excess blasts-1                                 | 21            | Favorable [116]    |
|                               | MDS with excess blasts-2                                 | 16            | Favorable [116]    |
|                               | MDS with isolated del(5q)                               | 16            | Unaffected [117]   |
|                               | MDS with Multilineage Dysplasia                          | 33            | Favorable [116]    |
|                               | MDS with Single Lineage Dysplasia                        | 20            | N/A                |
| MDS/MPN with Ring Sideroblasts and Thrombocytosis | MDS/MPN with Ring Sideroblasts and Thrombocytosis | 28            | N/A                |
|                               | MPN                                                      | 22            | Unaffected [118]   |
|                               | CMML                                                     | 56            | Favorable [118]    |
|                               | CML                                                      | 30            | Unaffected [119]   |
|                               | Essential thrombocytopenia                               | 9             | Unaffected [54]    |
|                               | Polycythemia Vera                                        | 28            | Unaffected [54]    |
|                               | Primary myelofibrosis                                    | 26            | Unaffected [54]    |
|                               | Histiocytic and Dendritic Cell Neoplasms                 | 2             | N/A                |
Table 1. Cont.

| Cancer Type | Detailed Cancer Type | Frequency (%) | Prognosis |
|-------------|----------------------|---------------|-----------|
| B/T-cell neoplasms (343/3712) 9% | Burkitt Lymphoma | 4 | N/A |
| | DLBCL, NOS | 6 | N/A |
| | DLBCL (unspecified) | 11 | Favorable [120] |
| | DLBCL, Germinal Center B-Cell Type | 7 | N/A |
| | DLBCL, Activated B-cell Type | 1 | N/A |
| | Follicular Lymphoma | 4 | N/A |
| | High-Grade B-Cell Lymphoma, NOS | 5 | N/A |
| | Mantle Cell Lymphoma | 3 | N/A |
| | Marginal Zone Lymphoma | 4 | N/A |
| | Mature B-Cell Neoplasms | 16 | N/A |
| | AITL | 78 | Unaffected [108] |
| | CLL/SLL | 1 | N/A |
| | Sezary Syndrome | 12 | N/A |
| Therapy-Related Neoplasms (12/115) 10% | Therapy-Related Myeloid Neoplasms (unspecific) | 8 | N/A |
| | Therapy-Related Myelodysplastic Syndrome | 27 | N/A |

4.1. Preleukemic Conditions

HSCs, like any other somatic cells, slowly accumulate mutations during the whole life of an individual [121]. TET2 mutated HSC clones are frequently found among healthy aged individuals characterized by an increased risk of cancer onset and a higher propensity to develop cardiovascular diseases [18,122–124]. This condition is named clonal hematopoiesis of indeterminate potential (CHIP). Mechanistically, TET2 loss of function mutations appear to enhance HSCs’ expansion capabilities and lead to a myeloid bias [48,52,125–127]. Additionally, TET2 mutant cells show an enhanced response to pro-inflammatory cytokines such as IL-6 and TNF-α [128], potentially representing a competitive advantage in response to inflammatory environments. Moreover, inflammation-based preleukemic myeloproliferation has been linked to TET2 activity [129,130], where Il6 expression might be negatively regulated by Tet2-mediated Hdac2 recruitment to its promoter [87].

Regardless, HSCs with altered TET2 activity acquire a competitive clonal advantage due to an altered DNA methylation landscape that finally allows aberrant gene expression. However, the precise order of events leading from CHIP to the development of myeloid malignancies is not yet fully uncovered, nor is the CHIP potential as a clinical predictor.

4.2. Leukemic Conditions

As previously mentioned, TET2 loss of function mutations are frequently found in patients with myeloid malignancies, myeloproliferative disorders (MPN), or myelodysplastic syndromes (MDS) (Table 1). These conditions are typically considered as an early event in myeloid cancer development [15,131] and are characterized by presenting aberrant DNA methylation profiles due to incorrect TET2 function [52,103]. This is the case, for instance, of chronic myelomonocytic leukemia (CMML), a mixed MDS/MPN neoplasm where TET2 mutations are present in up to 60% of patients [16,132] (Table 1). In CMML cells, aberrant methylation was observed at promoters of genes involved in neoplastic transformation, WNT and PDGF signaling pathways; inflammation; and apoptosis [133]. Of note, similar gene sets were observed aberrantly methylated and expressed in TET2-mutated AML patients, including genes coding for tumor suppressors (PDZD2); transcription modulators (ZNF667, ZNF582, PIAS2, CDK8); nuclear import receptors (TNPO3, IPO8); and myeloid cytokines (CSDA) [103].

Global methylation analysis also revealed a shared hypermethylation signature in a subset of AML patients carrying IDH1/2 mutations [52,134], a well-known TET2 inhibitor and mutually exclusive mutated gene (See Section 2.4). The latter highlights the importance of TET2 enzymatic deregulation.
Similarly, mutations in the IDH1/2-TET2-WT1 network, which collectively appear in 30-50% of AML cases [20,81,131], also present an apparent hypermethylated phenotype as a consequence of deficient TET2 activity [82,135]. Several ongoing clinical trials aim to restore TET2 activity within the IDH1/2-TET2-WT1 mutated network by combining hypomethylating agents with ascorbate treatment [50,51,135–137] (ClinicalTrials.gov identifier: NCT03999723, NCT03682029).

In the lymphoid lineage, aberrant B cell differentiation and chronic lymphocytic leukemia (CLL) have been associated with DNA hypomethylation [138–140]. Hypomethylation at gene bodies and enhancer regions correlates with gene expression differences in CLL samples compared to normal B cells [89,139,141]. However, available data do not support a primary role of TET enzymes in CLL, but perhaps an accessory role in establishing leukemia-specific patterns of 3D chromatin conformation. The latter might be accomplished, for instance, by modulating CTCF binding to the chromatin, a DNA methylation-sensitive mechanism [142].

TET2 has also been studied in the context of lymphoproliferative diseases. Diffuse large B-cell lymphoma (DLBCL) patients have shown specific hypermethylation signatures on promoters of tumor suppressor genes involved in cell fate and cell cycle changes [109]. Germinal center analysis of B cells in Tet2 deficient mice showed promoter hypermethylation and defective transcription factor binding at essential B-cell pathway genes. Tet2 knockout mice partially phenocopied DLBCL patients, characterized by downregulation of antigen presentation genes/interferon pathway, lymphoma-like transcriptional profiles similar to CREBBP-mutant patients, and a failure at the germinal center exit. Therefore, this data suggests that TET2 is relevant in B cell lymphoma development and how acquiring mutations in HSCs might influence B-cell maturation and cancer development [105,106].

5. Summary and Conclusions

The last decade of research has shed light on the complex biological functions of TET2 in normal hematopoiesis and blood cancer development. However, as described in this manuscript, a holistic approach is required to deconvolute the intricated regulatory networks determining TET2 function in a cell type-specific manner. Precisely, TET2 activity is controlled by: (1) TET2 transcriptional regulation (through Oct4, C/EBPα, Rinf or HDAC4); (2) post-transcriptional regulation (by miR-22, miR-9, or miR-34a); (3) post-translational regulation (by a whole array of PTMs (Figure 1); (4) enzymatic regulation (by VitC, α-KG/2-HG, FH, SDH or oxygen/HIF1α) and (5) genomic localization (through interactions with an extensive network of partners (Figure 2).

C/EBPα regulating Tet2 gene expression by directly recruiting Tet2 enzyme to demethylate its own enhancers during myeloid cell fate specification [10] perfectly illustrates the complexity of the Tet2 regulatory networks. Another interesting example of multilayer regulation is the miR-9/miR-34a-SIRT1-TET2 network. Sun and co-workers identified miR-9/miR-34a overexpression within a subset of TET2-WT MDS patients displaying a TET2 loss of function phenotype [32]. miR-9/miR-34a post-transcriptionally regulates SIRT1, which modulates TET2 activity by mediating deacetylation at key lysine residues of the enzyme [32].

About TET2 post-translational regulation, a comprehensive characterization of all potential PTMs described (Figure 1) is needed to fully determine their physiological relevance. Enhanced knowledge of TET2’s PTM-ome might be particularly relevant to fully elucidate the functions mediated by well-known TET2 interactors such as SIRT1 or OGT. Factors that can modify TET2 enzymatic capabilities and modulate its interaction with other proteins [32,37–40,42,43] (Figure 1).

Substrate availability is the primary mechanism of enzymatic regulation. Therefore, TET2 activity is tightly regulated by the availability of α-KG and 2-HG produced by IDH1/2-WT or -mutated enzymes, respectively [50,51]. First studies conducted in AML patient cells harboring 2-HG-producing IDH1/2 mutations uncovered an altered cellular epigenetic landscape and a myeloid bias characteristic of AML patients with TET2 loss
of function [52,90,134,143]. However, such myeloid bias has not been observed when analyzing the multilayer differentiation potential of Idh2 mutated cells through a single-cell approach [144]. The apparent discrepancy observed in the phenotype might be explained by species-specific differences, variations in 2-HG levels produced; differences in 2-HG subtype (D-2-HG or L-2-HG) produced; or a side-effect of 2-HG-mediated inactivation of other α-KG-dependent dioxygenases such as the Jumonji-C domain histone demethylase family.

The rapidly increasing list of partners/interactors identified highlights the idea that TET2 has been promiscuously recruited to the genome by sequence-specific transcription factors (also by other epigenetic regulators including HDAC1/2 or DNMT1) to drive active DNA demethylation in a cell state-specific manner. TET2 promiscuous behavior might be encoded in its nucleotide sequence where only part of the catalytic domain (coding for the cysteine-rich domain and the first double-stranded β-helix) is structured and ordered (Figures 1 and 2 and in silico prediction). Whereas the N-terminus protein region is unstructured, therefore, constituting a large intrinsically disordered domain (IDR) that might favor most TET2 described interactions through a mechanism of liquid-liquid phase separation (LLPS). Of note, IDR-mediated LLPS events have been recently described for the TET2 recruiter KLF4 and its associated pluripotency transcription factor OCT4 [145,146].

To sum up, fine dissection of the molecular mechanisms underlying TET2 activity is essential to fully uncover its contribution to clonal expansion and malignant transformation.

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References
1. Bird, A. DNA methylation patterns and epigenetic memory. Genes Dev. 2002, 16, 6–21. [CrossRef] [PubMed]
2. Baylin, S.B.; Jones, P.A. A decade of exploring the cancer epigenome—Biological and translational implications. Nat. Cancer 2011, 11, 726–734. [CrossRef] [PubMed]
3. Hackett, J.A.; Surani, M.A. Regulatory Principles of Pluripotency: From the Ground State Up. Cell Stem Cell 2014, 15, 416–430. [CrossRef] [PubMed]
4. Wu, X.; Zhang, X.W.Y. TET-mediated active DNA demethylation: Mechanism, function and beyond. Nat. Rev. Genet. 2017, 18, 517–534. [CrossRef] [PubMed]
5. Tahiliani, M.; Koh, K.P.; Shen, Y.; Pastor, W.A.; Bandukwala, H.; Brudno, Y.; Iyer, L.M.; Liu, D.R.; Aravind, L.; et al. Conversion of 5-Methylcytosine to 5-Hydroxymethylcytosine in Mammalian DNA by MLL Partner TET1. Science 2009, 324, 930–935. [CrossRef]
6. Ito, S.; D’Alessio, A.C.; Taranova, O.V.; Hong, K.; Sowers, L.C.; Zhang, Y. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature 2010, 466, 1129–1133. [CrossRef]
7. Ito, S.; Shen, L.; Dai, Q.; Wu, S.C.; Collins, L.B.; Swenberg, J.A.; He, C.; Zhang, Y. Tet Proteins Can Convert 5-Methylcytosine to 5-Formylcytosine and 5-Carboxylycytosine. Science 2011, 333, 1300–1303. [CrossRef]
8. Spruijt, C.G.; Gnerlich, F.; Smits, A.H.; Pfaffeneder, T.; Jansen, P.W.T.C.; Bauer, C.; Munzel, M.; Wagner, M.; Muller, M.; Khan, F.; et al. Dynamic readers for 5-(hydroxy) methylcytosine and its oxidized derivatives. Cell 2013, 152, 1146–1159. [CrossRef]
9. Kunimoto, H.; Fukuchi, Y.; Sakurai, M.; Takubo, K.; Okamoto, S.; Nakajima, H. Tet2-mutated myeloid progenitors possess aberrant in vitro self-renewal capacity. Blood 2014, 123, 2897–2899. [CrossRef]
10. Sardina, J.L.; Collombet, S.; Tian, T.; Gómez, A.; Di Stefano, B.; Berenguer, C.; Brumbaugh, J.; Stadhouders, R.; Morales, C.S.; Gut, M.; et al. Transcription Factors Drive Tet2-Mediated Enhancer Demethylation to Reprogram Cell Fate. Cell Stem Cell 2018, 23, 727–741.e9. [CrossRef]
11. Tsagaratou, A.; Lio, C.W.J.; Yue, X.; Rao, A. TET Methylcytosine Oxidases in T Cell and B Cell Development and Function. Frontiers in Immunol. 2017, 8, 220. [CrossRef] [PubMed]
12. Zhao, Z.; Chen, L.; Dawlaty, M.M.; Pan, F.; Weeks, O.; Zhou, Y.; Cao, Z.; Shi, H.; Wang, J.; Lin, L.; et al. Combined Loss of Tet1 and Tet2 Promotes B Cell, but Not Myeloid Malignancies, in Mice. Cell Rep. 2015, 13, 1692–1704. [CrossRef] [PubMed]
13. Cimmino, L.; Dawlaty, M.M.; Ndiaye-Lobry, D.; Yap, Y.S.; Bakogianni, S.; Yu, Y.; Bhattacharyya, S.; Shaknovich, R.; Geng, H.; Lobry, C.; et al. TET1 is a tumor suppressor of hematopoietic malignancy. Nat. Immunol. 2015, 16, 653–662. [CrossRef] [PubMed]
14. Poole, C.J.; Lodh, A.; Choi, J-H.; Van Riggelen, J. MYC deregulates TET1 and TET2 expression to control global DNA (hydroxy)methylation and gene expression to maintain a neoplastic phenotype in T-ALL. *Epigenet. Chromatin* **2019**, 12, 1–20. [CrossRef] [PubMed]

15. Delhommeau, F.; Dupont, S.; Della Valle, V.; James, C.; Trannoy, S.; Massé, A.; Kosmider, O.; Le Couedic, J-P.; Robert, F.; Alberdi, A.; et al. Mutation in TET2 in Myeloid Cancers. *N. Engl. J. Med.* **2009**, 360, 2289–2301. [CrossRef]

16. Kosmider, O.; Gelsi-Boyer, V.; Ciudad, M.; Raceour, C.; Jostee, V.; Ney, N.; Quesnel, B.; Fenaux, P.; Bastie, J-N.; Beyne-Rauzy, O.; et al. TET2 gene mutation is a frequent and adverse event in chronic myelomonocytic leukemia. *Haematologica* **2009**, 94, 1676–1681. [CrossRef]

17. Langemeijer, S.M.C.; Kuiper, R.P.; Berends, M.; Knops, R.; Aslanyan, M.G.; Massop, M.; Stevens-Linders, E.; Van Hoogen, P.; Van Kessel, A.G.; Raymakers, R.A.P.; et al. Acquired mutations in TET2 are common in myelodysplastic syndromes. *Nat. Genet.* **2009**, 41, 838–842. [CrossRef]

18. Busque, L.; Patel, J.P.; Figueroa, M.E.; Vasanthakumar, A.; Provost, S.; Hamilou, Z.; Mollica, L.; Li, J.; Viale, A.; Heguy, A.; et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat. Genet.* **2012**, 44, 1179–1181. [CrossRef]

19. Metzeler, K.H.; Maharry, K.; Radmacher, M.D.; Mršić, A.; Krugers, T.; Ylstra, B.; Pedersen, F.S.; Hasemann, M.S.; Damgaard, I.; Schuster, M.B.; Theilgaard-Mönch, K.; Sorensen, A.; et al. TET2 Mutations Improve the New European LeukemiaNet Risk Classification of Acute Myeloid Leukemia: A Cancer and Leukemia Group B Study. *J. Clin. Oncol.* **2011**, 29, 1373–1381. [CrossRef] [PubMed]

20. The Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *Cell Rep.* **2015**, 13, e2480–e2490. [CrossRef] [PubMed]

21. Hu, L.; Li, Z.; Cheng, J.; Rao, Q.; Gong, W.; Liu, M.; Shi, Y.G.; Zhu, J.; Wang, P.; Xu, Y. Crystal Structure of TET2-DNA Complex: Insight into TET-Mediated 5mC Oxidation. *Cell 2013*, 155, 1545–1555. [CrossRef]

22. Montagner, S.; Leoni, C.; Emming, S.; Della Chiara, G.; Balestrieri, C.; Barozzi, I.; Piccolo, V.; Togher, S.; Ko, M.; Rao, A.; et al. TET2 Regulates Mast Cell Differentiation and Proliferation through Catalytic and Non-catalytic activities. *Cell Rep.* **2016**, 15, 1566–1579. [CrossRef] [PubMed]

23. Ito, K.; Lee, J.; Chrysanthou, S.; Zhao, Y.; Josephs, K.; Sato, H.; Teruya-Feldstein, J.; Zheng, D.; Dawlaty, M.M.; Ito, K. Non-catalytic Roles of Tet2 Are Essential to Regulate Hematopoietic Stem and Progenitor Cell Homeostasis. *Cell Rep.* **2019**, 28, 2480–2490.e4. [CrossRef] [PubMed]

24. Koh, K.P.; Yabuuchi, A.; Rao, S.; Huang, Y.; Cunniff, K.; Nardone, J.; Laiho, A.; Tahiliani, M.; Sommer, C.A.; Mostoslavsky, G.; et al. Tet1- and Tet2-Mediated Foxp3 Demethylation to Drive Regulatory T Cell Differentiation and Maintain Immune Homeostasis. *Cell Stem Cell* **2013**, 13, 200–213. [CrossRef] [PubMed]

25. Ravichandran, M.; Lei, R.; Tang, Q.; Zhao, Y.; Lee, J.; Ma, L.; Chrysanthou, S.; Lorton, B.M.; Cvekl, A.; Shechter, D.; et al. Rnf15 Regulates Pluripotency Network Genes and Tet Enzymes in Embryonic Stem Cells. *Cell Rep.* **2019**, 28, 1993–2003.e5. [CrossRef] [PubMed]

26. Kallin, E.M.; Rodriguez-Uborea, J.; Christensen, J.; Cimmino, L.; Afantitis, I.; Helin, K.; Ballestar, E.; Graf, T. Tet2 Facilitates the Derepression of Myeloid Target Genes during CEBPα-Induced Transdifferentiation of Pre-B Cells. *Mol. Cell* **2012**, 48, 266–276. [CrossRef] [PubMed]

27. Hasemann, M.S.; Damgaard, I.; Schuster, M.B.; Theilgaard-Mönch, K.; Sorensen, A.; Mršić, A.; Krugers, T.; Ylstra, B.; Pedersen, F.S.; Nerlov, C.; et al. Mutation of C/EBPα predisposes to the development of myeloid leukemia in a retroviral insertional mutagenesis screen. *Blood 2008*, 111, 4309–4321. [CrossRef] [PubMed]

28. Huang, F.; Sun, J.; Chen, W.; He, X.; Zhu, Y.; Dong, H.; Wang, H.; Li, Z.; Zhang, L.; Khaled, S.; et al. HDAC4 inhibition disrupts TET2 function in high-risk MDS and AML. *Aging Cell* **2020**, 12, 16759–16774. [CrossRef]

29. Yang, R.; Qu, C.; Zhou, Y.; Konkel, J.E.; Shi, S.; Liu, Y.; Chen, C.; Liu, S.; Liu, D.; Chen, Y.; et al. Hydrogen Sulfide Promotes Tet1- and Tet2-Mediated Foxp3 Demethylation to Drive Regulatory T Cell Differentiation and Maintain Immune Homeostasis. *Immunity 2015*, 43, 251–263. [CrossRef]

30. Cheng, J.; Guo, S.; Chen, S.; Mastriano, S.J.; Liu, C.; D’Alessio, A.C.; Hysolli, E.; Guo, Y.; Yao, H.; Megyola, C.M.; et al. An Extensive Network of TET2-Targeting MicroRNAs Regulates Malignant Hematopoiesis. *Cell Rep.* **2013**, 5, 471–481. [CrossRef]

31. Song, S.J.; Ito, K.; Ala, U.; Kats, L.; Webster, K.; Sun, S.M.; Jongen-Lavrencic, M.; Manova-Todorova, K.; Teruya-Feldstein, J.; Avigan, D.E.; et al. The Oncogenic MicroRNA miR-22 Targets the TET2 Tumor Suppressor to Promote Hematopoietic Stem Cell Self-Renewal and Transformation. *Cell Stem Cell* **2013**, 13, 87–101. [CrossRef]

32. Sun, J.; He, X.; Zhu, Y.; Ding, Z.; Dong, H.; Feng, Y.; Du, J.; Wang, H.; Wu, X.; Zhang, L.; et al. SIRT1 Activation Disrupts Maintenance of Myelodysplastic Syndrome Stem and Progenitor Cells by Restoring TET2 Function. *Cell Stem Cell* **2018**, 23, 355–369.e9. [CrossRef] [PubMed]

33. Ko, M.; An, J.; Bandukwala, H.S.; Chavez, L.; Áijö, T.; Pastor, W.A.; Segal, M.F.; Li, H.; Koh, K.P.; Lähdesmäki, H.; et al. Modulation of TET2 expression and 5-methylcytosine oxidation by the CXCC domain protein IDAX. *Nature 2013*, 497, 122–126. [CrossRef] [PubMed]

34. Wang, Y.; Zhang, Y. Regulation of TET Protein Stability by Calpains. *Cell Rep.* **2014**, 6, 278–284. [CrossRef] [PubMed]
56. He, P.; Lei, J.; Zou, L.-X.; Zhou, G.-Z.; Peng, L.; Deng, Q.; Liu, X.-L. Effects of hypoxia on DNA hydroxymethylase Tet methylcytosine dioxygenase 2 in a KG-1 human acute myeloid leukemia cell line and its mechanism. Oncol. Lett. 2021, 22, 1–12. [CrossRef] [PubMed]

57. Jumper, J.; Evans, R.; Pritzel, A.; Green, T.; Figurnov, M.; Ronneberger, O.; Tunyasuvunakool, K.; Bates, R.; Žídek, A.; Potapenko, A.; et al. Highly accurate protein structure prediction with AlphaFold. Nature 2021, 596, 583–589. [CrossRef]

58. Varadi, M.; Anyango, S.; Deshpande, M.; Nair, S.; Natassia, C.; Yordanova, G.; Yuan, D.; Stroe, O.; Wood, G.; Laydon, A.; et al. AlphaFold Protein Structure Database: Massively expanding the structural coverage of protein-sequence space with high-accuracy models. Nat. Struct. Mol. Biol. 2021, 28, D439–D449. [CrossRef] [PubMed]

59. Di Stefano, B.; Collombet, S.; Jakobsen, J.S.; Wierer, M.; Sardina, J.L.; Lackner, A.; Stathouders, R.; Morales, C.S.; Francesconi, M.; Limone, F.; et al. C/EβPα creates elite cells for iPSC reprogramming by upregulating Klf4 and increasing the levels of Lsd1 and Brd4. Nat. Cell Biol. 2016, 18, 371–381. [CrossRef]

60. Di Stefano, B.; Sardina, J.L.; van Oevelen, C.; Collombet, S.; Kallin, E.M.; Vicent, G.P.; Lu, J.; Thieffry, D.; Beato, M.; Graf, T. C/EβPα poises B cells for rapid reprogramming into induced pluripotent stem cells. Nature 2013, 506, 235–239. [CrossRef]

61. Piccolo, F.M.; Bagci, H.; Brown, K.E.; Landeira, D.; Soza-Ried, J.; Feytout, A.; Moolijman, D.; Hajkova, P.; Leitch, H.G.; Tada, T.; et al. Different Roles for Tet1 and Tet2 Proteins in Reprogramming-Mediated Erasure of Imprints Induced by EGC Fusion. Molecular Cell 2013, 49, 1023–1033. [CrossRef] [PubMed]

62. Costa, Y.; Ding, J.; Theunissen, T.W.; Faiola, F.; Hore, T.A.; Shliaha, P.V.; Fidalgo, M.; Saunders, A.; Lawrence, M.; Dietmann, S.; et al. NANOG-dependent function of TET1 and TET2 in establishment of pluripotency. Nature 2013, 495, 370–374. [CrossRef] [PubMed]

63. Hu, X.; Zhang, L.; Mao, S.-Q.; Li, Z.; Chen, J.; Zhang, R.-R.; Wu, H.-P.; Gao, J.; Guo, F.; Liu, W.; et al. Tet and TDG Mediate DNA Demethylation Essential for Mesenchymal-to-Epithelial Transition in Somatic Cell Reprogramming. Cell Stem Cell 2014, 14, 512–522. [CrossRef] [PubMed]

64. Guallar, D.; Bi, X.; Pardavila, J.A.; Huang, X.; Saenz, C.; Shi, X.; Zhou, H.; Faiola, F.; Ding, J.; Haruehanroengra, P.; et al. RNA-dependent chromatin targeting of TET2 for endogenous retrovirus control in pluripotent stem cells. Nat. Genet. 2018, 50, 443–451. [CrossRef] [PubMed]

65. Dai, H.-Q.; Wang, B.-A.; Yang, L.; Chen, J.-J.; Zhu, G.-C.; Sun, M.-L.; Ge, H.; Wang, R.; Chapman, D.; Tang, F.; et al. Early-stage epigenetic modification during somatic cell reprogramming by Parp1 and Tet2. Nature 2012, 488, 652–655. [CrossRef] [PubMed]

66. Fidalgo, M.; Huang, X.; Guallar, D.; Sanchez-Priego, C.; Valdes, V.J.; Saunders, A.; Ding, J.; Wu, W.-S.; Clavel, C.; Wang, J. Zip281 Coordinates Opposing Functions of Tet1 and Tet2 in Pluripotent States. Cell Stem Cell 2016, 19, 355–369. [CrossRef] [PubMed]

67. Pantier, R.; Tatar, T.; Colby, D.; Chambers, I. Endogenous epitope-tagging of Tet1, Tet2 and Tet3 identifies TET2 as a naïve pluripotency marker. Life Sci. Aliments 2019, 2, e201900516. [CrossRef] [PubMed]

68. Guallar, D.; Bi, X.; Pardavila, J.A.; Huang, X.; Saenz, C.; Shi, X.; Zhou, H.; Faiola, F.; Ding, J.; Haruehanroengra, P.; et al. TET1-dependent function of UTX in establishment of pluripotency. Nat. Genet. 2020, 52, 819–827. [CrossRef] [PubMed]

69. Potapenko, A.; Orito, E.; Matsui, A.; van Es, K.; Beppu, T.; Melton, D.A.; Orkin, S.H.; Maitra, A.; et al. PRDM14 promotes active DNA demethylation through the Ten-eleven translocation (TET)-mediated base modification pathway. Nature 2014, 512–522. [CrossRef] [PubMed]

70. Okashita, N.; Kumaki, Y.; Ebi, K.; Nishi, M.; Okamoto, Y.; Nakayama, M.; Hashimoto, S.; Nakamura, T.; Sugasawa, K.; Macfarlan, T.S.; Gifford, W.D.; Driscoll, S.; Lettieri, K.; Rowe, H.M.; Bonanomi, D.; Firth, A.; Singer, O.; Trono, D.; Pfaff, S.L. Embryonic stem cell potency fluctuates with endogenous retrovirus activity. Nature 2012, 487, 57–63. [CrossRef]

71. Bar-Nur, O.; Brumbaugh, J.; Verheul, C.; Apostolou, E.; Pruteanu-Malinici, I.; Walsh, R.M.; Ramaswamy, S.; Hochdelinger, K. Small molecules facilitate rapid and synchronous iPSC generation. Nat. Chem. Biol. 2014, 11, 1170–1176. [CrossRef]

72. Smith, Z.D.; Shi, J.; Wu, H.; Donaghey, J.; Clement, K.; Caciarelli, D.; Girkar, A.; Michor, F.; Meissner, A. Epigenetic restriction of extraembryonic lineages mirrors the somatic transition to cancer. Nature 2017, 549, 543–547. [CrossRef] [PubMed]

73. Zhang, D.-E.; Zhang, P.; Wang, N.-D.; Hetherington, C.J.; Darlington, G.J.; Tenen, D.G. Absence of granulocyte colony-stimulating factor signaling and neutrophil development in CCAAT enhancer binding protein-deficient mice. Proc. Natl. Acad. Sci. USA 1997, 94, 569–574. [CrossRef] [PubMed]
99. Chen, L.-L.; Lin, H.-P.; Zhou, W.-J.; He, C.-X.; Zhang, Z.-Y.; Cheng, Z.-L.; Song, J.-B.; Liu, P.; Chen, X.-Y.; Xia, Y.-K.; et al. SNIP1 regulates site specificity of DNA demethylation by recruitment of DNA demethylation machineries in hematopoietic cells. Blood Adv. 2017, 1, 1699–1711. [CrossRef] [PubMed]

100. Song, C.; Wang, L.; Wu, X.; Wang, K.; Xie, D.; Xiao, Q.; Li, S.; Jiang, K.; Liao, L.; Yates, J.R.; et al. PML recruits HDAC2 to specifically repress IL-6. Cancer Res. 2015, 75, 3288–3297. [CrossRef] [PubMed]

101. Rasmussen, K.D.; Jia, G.; Johansen, J.V.; Pedersen, M.T.; Rapin, N.; Bagger, F.O.; Porse, B.T.; Bernard, O.A.; Christensen, J.; Helin, K. Loss of TET2 in hematopoietic cells leads to DNA hypermethylation of active enhancers and induction of leukemogenesis. Genes Dev. 2015, 29, 910–922. [CrossRef] [PubMed]
Cancers 2022, 14, 830

102. Tulstrup, M.; Soerensen, M.; Hansen, J.W.; Gillberg, L.; Needham, M.; Kastrup, K.; Helin, K.; Christensen, K.; Weischenfeldt, J.; Grønbæk, K. TET2 mutations are associated with hypermethylation at key regulatory enhancers in normal and malignant hematopoiesis. Nat. Commun. 2021, 12, 1–10. [CrossRef]

103. Figueroa, M.E.; Lugthart, S.; Li, Y.; Erpelink-Verschueren, C.; Deng, X.; Christos, P.J.; Schifano, E.; Booth, J.; van Putten, W.; Skrabanek, L.; et al. DNA Methylation Signatures Identify Biologically Distinct Subtypes in Acute Myeloid Leukemia. Cancer Cell 2010, 17, 13–27. [CrossRef]

104. Ko, M.; Huang, Y.; Jankowska, A.M.; Pape, U.J.; Tahiliani, M.; Bandukwala, H.S.; An, J.; Lamperti, E.D.; Koh, K.P.; Ganetguelin, W.; et al. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. Nature 2010, 468, 839–843. [CrossRef]

105. Dominguez, P.M.; Ghamlouch, H.; Rosikiewicz, W.; Kumar, P.; Béguélin, W.; Fontan, L.; Rivas, M.A.; Pawlikowska, P.; Armand, M.; Mously, E.; et al. TET2 deficiency causes germinal center hyperplasia, impairs plasma cell differentiation and promotes B-cell lymphomagenesis. Cancer Discov. 2018, 8, 1632–1653. [CrossRef] [PubMed]

106. Rosikiewicz, W.; Chen, X.; Dominguez, P.M.; Ghamlouch, H.; Aoufouchi, S.; Bernard, O.A.; Melnick, A.; Li, S. TET2 deficiency reprograms the germinal center B cell epigenome and silences genes linked to lymphomagenesis. Sci. Adv. 2020, 6, eaay5872. [CrossRef]

107. Couronné, L.; Bastard, C.; Bernard, O.A. TET2 and DNMT3A Mutations in Human T-Cell Lymphoma. N. Engl. J. Med. 2012, 366, 95–96. [CrossRef]

108. Odejede, O.; Weigert, O.; Lane, A.A.; Toscano, D.; Lunning, M.A.; Kopp, N.; Kim, S.S.; Van Bodegom, D.; Bolla, S.; Schatz, J.; et al. A targeted mutational landscape of angioimmunoblastic T-cell lymphoma. Blood 2014, 123, 1293–1296. [CrossRef]

109. Asmar, F.; Punj, V.; Christensen, J.; Pedersen, M.T.; Pedersen, A.; Nielsen, A.B.; Høcher, C.; Ralfkiaer, U.; Brown, P.; Ralfkiaer, E.; et al. Genome-wide profiling identifies a DNA methylation signature that associates with TET2 mutations in diffuse large B-cell lymphoma. Haematologica 2013, 98, 1912–1920. [CrossRef]

110. Coltro, G.; Mangaonkar, A.A.; Laslo, T.L.; Finke, C.M.; Pophali, P.; Carr, R.; Gangat, N.; Binder, M.; Pardanani, A.; Fernandez-Zapico, M.; et al. Clinical, molecular, and prognostic correlates of number, type, and functional localization of TET2 mutations in chronic myelomonocytic leukemia (CMML)—A study of 1084 patients. Leukemia 2020, 34, 1407–1421. [CrossRef] [PubMed]

111. Lewis, N.E.; Petrova-Drus, K.; Huet, S.; Epstein-Peterson, Z.D.; Gao, Q.; Sigler, A.E.; Baik, J.; Ozkaya, N.; Moskowitz, A.J.; Kumar, A.; et al. Clonal hematopoiesis in angioimmunoblastic T-cell lymphoma with divergent evolution to myeloid neoplasms. Blood Adv. 2020, 4, 2261–2271. [CrossRef] [PubMed]

112. Yao, W.; Wu, F.; Zhang, W.; Chuang, S.; Thompson, J.S.; Chen, Z.; Zhang, S.; Clipson, A.; Wang, M.; Liu, H.; et al. Angioimmunoblastic T-cell lymphoma contains multiple clonal T-cell populations derived from a common TET2 mutant progenitor cell. J. Pathol. 2019, 250, 346–357. [CrossRef] [PubMed]

113. Wang, R.; Gao, X.; Yu, L. The prognostic impact of tet oncogene family member 2 mutations in patients with acute myeloid leukemia: A systematic-review and meta-analysis. BMC Cancer 2019, 19, 389. [CrossRef] [PubMed]

114. Konstandin, N.P.; Pastore, F.; Herold, T.; Dufour, A.; Rothenberg-Thurley, M.; Hinrichsen, T.; Ksienzyk, B.; Tschuri, S.; Schneider, S.; Hoster, E.; et al. Genetic heterogeneity of cytogenetically normal AML with mutations of CEBPA. Blood Adv. 2018, 2, 2724–2731.

115. Kosmidis, O.; Delabesse, E.; Mas, V.M.-D.; Cornillet-Lefebvre, P.; Blanchet, O.; Delmer, A.; Recher, C.; Raynaud, S.; Bouscary, D.; Vigué, F.; et al. TET2 mutations in secondary acute myeloid leukemias: A French retrospective study. Haematologica 2011, 96, 1059–1063. [CrossRef] [PubMed]

116. Kosmidis, O.; Gelsi-Boyer, V.; Cheok, M.; Grabar, S.; Della-Valle, V.; Picard, F.; Vigué, F.; Quesnel, B.; Beyne-Rauzy, O.; Solary, E.; et al. TET2 mutation is an independent favorable prognostic factor in myelodysplastic syndromes (MDS). Blood 2009, 114, 3285–3291. [CrossRef] [PubMed]

117. Mallo, M.; Del Rey, M.; Ibáñez, M.; Calasanz, M.J.; Arenillas, L.; Larráyoz, M.J.; Pedro, C.; Jerez, A.; Maciejewski, J.; Costa, D.; et al. Response to lenalidomide in myelodysplastic syndromes with del(5q): Influence of cytogenetics and mutations. Br. J. Haematol. 2013, 162, 74–86. [CrossRef]

118. Coltro, G.; Antelo, G.; Laslo, T.L.; Bs, C.M.F.; Pardanani, A.; Gangat, N.; Carr, R.M.; Binder, M.; Mangaonkar, A.A.; Ketterminger, R.; et al. Phenotypic correlates and prognostic outcomes of TET2 mutations in myelodysplastic syndrome/myeloproliferative neoplasm overlap syndromes: A comprehensive study of 504 adult patients. Am. J. Hematol. 2020, 95. [CrossRef]

119. Makishima, H.; Jankowska, A.M.; McDevitt, M.A.; O’Keefe, C.; Dujardin, S.; Cazzolli, H.; Przychodzen, B.; Prince, C.; Nicoll, J.; Siddaiah, H.; et al. CBL, CBLB, TET2, ASXL1, and IDH1/2 mutations and additional chromosomal aberrations constitute molecular events in chronic myelogenous leukemia. Blood 2011, 117, e198–e206. [CrossRef]

120. Lacy, S.E.; Barrans, S.L.; Beer, P.A.; Painter, D.; Smith, A.G.; Roman, E.; Cooke, S.L.; Ruiz, C.; Glover, P.; Van Hoppe, S.J.L.; et al. Targeted sequencing in DLBCL, molecular subtypes, and outcomes: A Haematological Malignancy Research Network report. Blood 2020, 135, 1759–1771. [CrossRef]

121. Welch, J.S.; Ley, T.J.; Link, D.C.; Miller, C.A.; Larson, D.E.; Koboldt, D.C.; Wartman, L.; Lamprecht, T.L.; Liu, F.; Xia, J.; et al. The Origin and Evolution of Mutations in Acute Myeloid Leukemia. Cell 2012, 150, 264–278. [CrossRef] [PubMed]

122. Jaiswal, S.; Fontanillas, P.; Flannick, J.; Manning, A.; Graumau, P.V.; Mar, B.G.; Lindsley, R.C.; Mermel, C.H.; Burtt, N.; Chavez, A.; et al. Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes. N. Engl. J. Med. 2014, 371, 2488–2498. [CrossRef]
123. Fuster, J.J.; MacLauchlan, S.; Zuriaga, M.A.; Polackal, M.N.; Ostriker, A.C.; Chakraborty, R.; Wu, C.-L.; Sano, S.; Muralidharan, S.; Rius, C.; et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. Science 2017, 355, 842–847. [CrossRef] [PubMed]

124. Xie, M.; Lu, C.; Wang, J.; McLellan, M.D.; Johnson, K.J.; Wendl, M.C.; McMichael, J.F.; Schmidt, H.K.; Yellapantula, V.; Miller, C.A.; et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat. Med. 2014, 20, 1472–1478. [CrossRef] [PubMed]

125. Li, Z.; Cai, X.; Cai, C.-L.; Wang, J.; Zhang, W.; Petersen, B.E.; Yang, F.-C.; Xu, M. Deletion of Dnmt3a and Tet2 Mutations on Hematopoietic Progenitor Cell Fitness. Stem Cell Rep. 2020, 14, 551–560. [CrossRef]

126. Ostrander, E.L.; Kramer, A.C.; Mallaney, C.; Celik, H.; Koh, W.K.; Fairchild, J.; Haussler, E.; Zhang, C.R.; Challen, G.A. Divergent Effects of Dnmt3a and Tet2 Mutations in Hematopoietic Progenitor Cells. Mol. Cell 2014, 53, 403–413. [CrossRef] [PubMed]

127. Rasmussen, K.D.; Berest, I.; Keßler, S.; Nishimura, K.; Simon-Morroni, S.; Vassiliou, G.S.; Pedersen, M.T.; Christensen, J.; Zaugg, J.B.; Heim, K. TET2 binding to enhancers facilitates transcription factor recruitment in hematopoietic cells. Genome Res. 2019, 29, 564–575. [CrossRef]

128. Cai, Z.; Kotzin, J.J.; Ramdas, B.; Chen, S.; Nelanuthala, S.; Palam, L.R.; Pandey, R.; Mali, R.S.; Liu, Y.; Kelley, M.R.; et al. Inhibition of Inflammatory Signaling in Tet2 Mutant Preleukemic Mitigates Stress-Induced Abnormalities and Clonal Hematopoiesis. Cell Stem Cell 2018, 23, 833–849.e5. [CrossRef]

129. Cull, A.H.; Snetsinger, B.; Buckstein, R.; Wells, R.A.; Rauh, M.J. Tet2 restrains inflammatory gene expression in macrophages. Exp. Hematol. 2017, 55, 66–70.e13. [CrossRef]

130. Meisel, M.; Hinterleitner, R.; Pacis, A.; Chen, L.; Earley, Z.M.; Mayassi, T.; Pierre, J.F.; Ernest, J.D.; Galipeau, H.J.; Thuille, N.; et al. Microbial signals drive pre-leukaemic myeloproliferation in a Tet2-deficient host. Nature 2018, 557, 580–584. [CrossRef]

131. Papaemmanuil, E.; Gerstung, M.; Bullinger, L.; Gaidzik, V.I.; Paschka, P.; Roberts, N.D.; Potter, N.E.; Heuser, M.; Thol, F.; Bolli, N.; et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. N. Engl. J. Med. 2016, 374, 2209–2221. [CrossRef] [PubMed]

132. Elena, C.; Galli’, A.; Such, E.; Meggendorfer, M.; Germing, U.; Rizzo, E.; Cervera, J.; Molteni, E.; Fasan, A.; Schuler, E.; et al. Integrating clinical features and genetic lesions in the risk assessment of patients with chronic myelomonocytic leukemia. Blood 2016, 128, 1408–1417. [CrossRef]

133. Palomo, L.; Malinverni, R.; Cabezón, M.; Xicoy, B.; Arnan, M.; Coll, R.; Pomares, H.; García, O.; Fuster-Tormo, F.; Grau, J.; et al. DNA methylation profile in chronic myelomonocytic leukemia associates with distinct clinical, biological and genetic features. Epigenetics 2018, 13, 8–18. [CrossRef]

134. Wilson, E.R.; Helton, N.M.; Heath, S.E.; Fulton, R.S.; Payton, J.E.; Welch, J.S.; Walter, M.J.; Westervelt, P.; DiPersio, J.F.; Link, D.C.; et al. Focal disruption of DNA methylation dynamics at enhancers in IDH-mutant AML cells. Nat. Genet. 2020, 52, 10–17. [CrossRef] [PubMed]

135. Das, A.; Kakadia, P.M.; Wojcik, D.; Pemberton, L.; Browett, P.J.; Bohlander, S.K.; Vissers, M.C.M. Clinical remission following ascorbate treatment in a case of acute myeloid leukaemia with mutations in TET2 and WT1. Blood Cancer J. 2019, 9, 1–5. [CrossRef] [PubMed]

136. Zhao, H.; Zhu, H.; Huang, J.; Zhu, Y.; Hong, M.; Zhu, H.; Zhang, J.; Li, S.; Yang, L.; Lian, Y.; et al. The synergy of Vitamin C with decitabine activates TET2 in leukemic cells and significantly improves overall survival in elderly patients with acute myeloid leukemia. Leuk. Res. 2018, 66, 1–7. [CrossRef] [PubMed]

137. Migay, M.; Chaturvedi, A.; Bilieny, M.; Cao, Q.; Jackson, L.; Hui, T.; Moks, M.; Heravi-Moussavi, A.; Humphries, R.K.; Heuser, M.; et al. Vitamin C-induced epigenomic remodelling in IDH1 mutant acute myeloid leukaemia. Leukemia 2017, 31, 11–20. [CrossRef] [PubMed]

138. Kulis, M.; Heath, S.; Bibikova, M.; Queirós, A.C.; Navarro, A.; Clot, G.; Martinez-Trillos, A.; Castellano, G.; Brun-Heath, I.; Pinyol, M.; et al. Epigenetic analysis detects gene-body DNA hypomethylation in chronic lymphocytic leukemia. Nat. Genet. 2012, 44, 1236–1242. [CrossRef]

139. Oakes, C.C.; Seifert, M.; Assenov, Y.; Gu, L.; Przekopowitz, M.; Ruppert, A.S.; Wang, Q.; Imbusch, C.D.; Serva, A.; Koser, S.D.; et al. DNA methylation dynamics during B cell maturation underlie a continuum of disease phenotypes in chronic lymphocytic leukemia. Nat. Genet. 2016, 48, 253–264. [CrossRef]

140. Beekman, R.; Chapaprieta, V.; Russiñol, N.; Vilarrasa-Blasi, R.; Verdaguer, N.; Martens, J.; Duran-Ferrer, M.; Kulis, M.; Serra, F.; Javierre, B.M.; et al. The reference epigenome and regulatory chromatin landscape of chronic lymphocytic leukemia. Nat. Med. 2018, 24, 868–880. [CrossRef] [PubMed]

141. Vilarrasa-Blasi, R.; Soler-Vila, P.; Verdaguer-Dot, N.; Russiñol, N.; Di Stefano, M.; Chapaprieta, V.; Clot, G.; Farabella, L.; Cuscó, P.; Kulis, M.; et al. Dynamics of genome architecture and chromatin function during human B cell differentiation and neoplastic transformation. Nat. Commun. 2021, 12, 1–18. [CrossRef] [PubMed]

142. Maurano, M.T.; Wang, H.; John, S.; Shafer, A.; Canfield, T.; Lee, K.; Statamotyanopoulos, J.A. Role of DNA Methylation in Modulating Transcription Factor Occupancy. Cell Rep. 2015, 12, 1184–1195. [CrossRef] [PubMed]

143. Sasaki, M.; Knobbe, C.B.; Mungar, J.C.; Lind, E.F.; Brenner, D.; Bruestle, A.; Harris, I.S.; Holmes, R.; Wakeham, A.; Haight, J.; et al. IDH1(R132H) mutation increases murine haematopoietic progenitors and alters epigenetics. Nature 2012, 488, 656–659. [CrossRef] [PubMed]

144. Izzo, F.; Lee, S.C.; Poran, A.; Chaligre, R.; Gaiti, F.; Gross, B.; Murali, R.R.; Deochand, S.D.; Ang, C.; Jones, P.W.; et al. DNA methylation disruption reshapes the hematopoietic differentiation landscape. Nat. Genet. 2020, 52, 378–387. [CrossRef]
145. Sharma, R.; Choi, K.-J.; Quan, M.D.; Sharma, S.; Sankaran, B.; Park, H.; LaGrone, A.; Kim, J.J.; MacKenzie, K.R.; Ferreon, A.C.M.; et al. Liquid condensation of reprogramming factor KLF4 with DNA provides a mechanism for chromatin organization. *Nat. Commun.* 2021, 12, 1–17. [CrossRef]

146. Boija, A.; Klein, I.A.; Sabari, B.R.; Dall’Agnese, A.; Coffey, E.L.; Zamudio, A.V.; Li, C.H.; Shrinivas, K.; Manteiga, J.C.; Hannett, N.M.; et al. Transcription Factors Activate Genes through the Phase-Separation Capacity of Their Activation Domains. *Cell* 2018, 175, 1842–1855.e16. [CrossRef] [PubMed]