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REVIEW

Polycomb group proteins and MYC: the cancer connection

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Abstract Polycomb group proteins (PcGs) are transcriptional repressors involved in physiological processes whereas PcG deregulation might result in oncogenesis. MYC oncogene is able to regulate gene transcription, proliferation, apoptosis, and malignant transformation. MYC deregulation might result in tumorigenesis with tumor maintenance properties in both solid and blood cancers. Although the interaction of PcG and MYC in cancer was described years ago, new findings are reported every day to explain the exact mechanisms and results of such interactions. In this review, we summarize recent data on the PcG and MYC interactions in cancer, and the putative involvement of microRNAs in the equation.

Keywords Polycomb group proteins · MYC · EZH2 · BMI1 · MicroRNA · Cancer

Polycomb group proteins

Polycomb group proteins (PcGs) represent transcriptional repressors of protein-coding gene promoters required for genomic imprinting, chromosome X inactivation (XCI), stem cell plasticity, cell fate determination, and maintenance of the correct cell identity at different developmental stages. Gene repression, which seems to be a dynamic process during differentiation, is achieved via PcG recruitment to target genes. That process involves several different transcription factors (TFs), long non-coding RNAs (lncRNAs), and fusion oncoproteins like PML/RARα and PLZF/RARα. PcGs are closely associated with DNA methylation by recruiting DNA methyltransferases (DNMTs) to target genes [1–6]. Recent findings suggest that XCI spreading seems to be governed by a hierarchy of two types of PcG target sites; the 'canonical' sites, which typically contain CpG islands and the 'non-canonical' sites that lack H3K4me3 or CpG islands. XCI requires a network involving the lncRNA XIST linked to the proteins HBiX and SMCHD1 [7, 8]. However, previously published data supported that PcG are not required for initiating or maintaining random XCI in mouse embryonic cells [9]. PcG have important regulatory functions during the cell cycle phases repressing cyclins, cyclin-dependent kinase (CDK) inhibitors, the pRB–E2F complex, while they can control DNA synthesis during S phase. PcG affect DNA damage pathways and repair mechanisms. They also regulate apoptosis and prevent the onset of senescence as PcG are downregulated during replicative senescence [10]. For instance, PcG proteins confer the hematopoietic stem cell (HSCs) the ability to act as progenitors and protects them from apoptosis through regulation of the *Ink4α/Arf* locus. Compositional changes of PcG proteins induces differentiation blockade, leading to malignant hematopoietic phenotypes [11, 12].

PcGs have the ability to re-establish the histone code on newly assembled unmethylated histones, at least in Drosophila [13]. They remain associated with mitotic chromatin in Drosophila S2 cells in order to ensure equal segregation

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to both new cells, or to keep local concentration of PcG proteins near the DNA at high levels, facilitating rebinding after mitosis [14].

PcG form multiprotein complexes called polycomb repressive complex 1 and 2 (PRC1/2) responsible for gene silencing through post-translational histone modifications. They are involved in monoubiquitylation of lysine 119 of histone H2A (H2AK119ub) and di- and tri-methylation of lysine 27 of histone H3 (H3K27me3), respectively. Multiple forms of the PRC1 exist containing combinations of different PcG proteins (CBX2/4/6/7/8), posterior sex combs proteins PCGF1 (NSPC1), PCGF2 (MEL18), PCGF3, PCGF4 (BMI1), PCGF5, PCGF6 (MBLR), the RING1 and RNF2 (RING2) proteins, three PH proteins (PHC1/2/3 or HPH1/2/3), two Sex combs on midleg (SMCL1/2), and RYBP/YAF2 [1, 15]. PRC2 consists of the core subunits enhancer of zeste 1/2 (EZH1/2), embryonic ectoderm development (EED), the RBBP7/4 (RbAp46/48) protein, and the suppressor of zeste 12 (SUZ12) [16]. Although EZH2 and EZH1 seem to be the catalytic subunit of PRC2, EED specifically recognizes and binds to the repressive trimethylated lysine marks contributing to the affinity of PRC2 for the nucleosomes [17]. PRC2 contains other proteins like AEBP2, PHF1 (PCL1), MTF2 (PCL2), PHF19 (PCL3), and JARID2, which regulate the enzymatic activity of PRC2 and co-localize with PRC2 on target genes. PRC2 is inhibited by H3K4me3 and H3K36me2/3, preventing H3K27me3 occupancy on transcriptionally active genes [18]. On the other hand, PRC1 might affect the ability of TF-IID to remain attached to gene promoters, suggesting a putative transcriptional silencing mechanism. In this way, target genes stay in a poised state to be expressed later during the developmental process [19]. Mounting evidence suggests that both PRC1 and PRC2 complexes can be recruited by lncRNAs on target genes promoting gene silencing [20].

The role of other histone modifications in PRC2 regulation is only now becoming apparent, and very recent data made evident that the member of the PCL family Phf19 regulates PRC2 occupancy. Phf19 recruits specific demethylases to mouse embryonic stem cells (mESCs) during differentiation leading to H3K27me3 and transcriptional silencing [21, 22]. PRC2 subunits might act either as a tumor suppressor (TS) or as an oncogene, suggesting that H3K27me3 may possess dual functions in different cell types [23]. Recent findings demonstrated that the PRC2 complex is allosterically activated by neighboring nucleosomes. Active genes are resistant to PRC2 activity, not only because of active epigenetic marks that antagonize PRC2 activity but also because the chromatin of transcribing genes is less compact, with lower nucleosome density. Therefore, the ability of PRC2 to distinguish active chromatin and dense inactive chromatin highlights the efficiency of PRC2 in maintaining the inactive states of target genes [24]. PHF1 has also been recently found to protect p53 from MDM2 degradation n vitro and in vivo [25].

BMI11 is probably the most studied subunit of PRC1, and is involved in normal stem cell proliferation affecting self-renewal and maintenance of HSCs and neuronal stem cells. It also has an important role in the malignant transformation of normal stem or differentiated cells in cancer stem cells. It is associated with metastasis and chemoresistence to agents routinely used in the battle against cancer. On the contrary, BMI1 silencing enhances the antitumor activity of chemotherapeutic agents [26, 27].

In physiological conditions, Ezh2 is essential for fetal hematopoiesis affecting erythroid differentiation in the fetal liver, while it does not seem to affect self-renewal in adult bone marrow. It might also epigenetically regulate transcriptional programs controlling migration and connectivity of neurons in the cortico–ponto–cerebellar pathway in mice [28, 29]. Ezh2 is closely associated with DNMTs as it is capable of recruiting DNMTs to gene promoters promoting gene silencing through DNA methylation [30].

Deregulated expression of PcG proteins has been identified in several types of hematologic malignancies and solid cancers via modulation of Notch, Hedgehog, and Wnt pathways [31–36]. Intriguingly, EZH2 oncogenic function has been demonstrated not to depend on gene silencing but rather on the co-activation of the transcriptional induction of its target genes in castration-resistant prostate cancer due to EZH2-phosphorylation in a PRC2-independent pattern [37].

An MYC overview

MYC proto-oncogene belongs to a family that includes the *c-MYC*, the *MYCL*, and the *MYCN* homologues, which share general topography. In normal cells, MYC expression is regulated at the transcriptional, post-transcriptional, translational, and post-translational levels. In order to activate gene transcription, MYC forms heterodimers and acts in conjunction with the MYC-associated protein X (MAX), required for regulation of gene transcription, proliferation, apoptosis, and malignant transformation. MYC deregulation has tumor initiating and tumor maintenance properties in both solid and blood cancers. The oncogenic activity is attributed to gene amplification, translocations, and to deregulation of its cofactors such as MAX. MYC acts downstream of receptor signaling pathways, including Wnt, Notch, phosphoinositide 3-kinase (PI3K), and Ras, and interacts with microRNAs (miRNAs), regulating transcription of genes involved in cell growth and proliferation [38–40].

C-MYC expression is high during early embryonic development and persists in the cell cycle phases, whereas it is low or even undetectable in differentiated tissues [41]. A rather unusual feature of MYC is that it can regulate all three RNA polymerases (RNA pol) for a subset of their respective targets. Thus, besides protein-coding genes and many non-coding RNAs, including miRNA and lncRNA genes that are controlled by Pol II, MYC regulates rRNA and tRNA genes that are transcribed by Pol I and Pol III, respectively [42]. MYC exhibit antisenescence functions through its interaction with *CDK2* or by repressing cell-cycle inhibitors like $p15^{ink4b}$, $p21^{cip1}$, activates genes involved in cell cycle progression (*CDK4*, *cyclin D1*), and possesses immunomodulatory potential by preventing T-cells from recognizing tumor cells [43, 44].

Lin et al. [45] have recently demonstrated that, in different tumor cells bearing high c-MYC levels, it occupies sites at both promoters and enhancers of the actively transcribed genes across the cancer cell genome. The increase in c-MYC occupancy leads to increased transcription elongation by RNA Pol II and increased levels of transcripts per cell. In that way, c-MYC causes transcriptional amplification, producing elevated levels of transcripts from the existing gene-expression program of tumor cells potentiating the already deregulated transcriptional program of cancer cells. Another intriguing MYC characteristic is that aside from inducing cell proliferation, it can also enhance programmed cell death and paradoxically might induce carcinogenesis by enhancing compensatory proliferation [46]. Myc causes malignant transformation of the cell but it can also induce apoptotic cell death through still unclear mechanisms. It seems that the TS *Arf* mediates such a switch via inhibition of the ubiquitylation of the c-Myc transcriptional domain. In turn, such inhibition induces the *Egr1* gene essential for the c-Myc-induced p53-independent apoptosis observed in double knock out experiments in mouse embryonic fibroblasts (MEFs). That interaction is deregulated in cancer, through the overexpression of Skp2, which inhibits the recruitment of Arf to Egr1 [47].

Myc regulates global chromatin structure contributing to oncogenesis through histone modifications targeting the epigenetic machinery [48]. In mouse naïve cells, Myc binding sites are characterized by active chromatin marks while they are excluded from regions with repressive marks [49]. Target gene repression may be indirect since transcriptional repressors activated by Myc are recruited to Myc target genes as in the case of *Ezh2*. *Ezh2* upregulation during B-cell activation mediates transcriptional repression across the genome via H3K27me3. Overall, the findings of that elegant study determine that Myc is a universal amplifier of gene activation affecting multiple alternative pathways [50].

Here, we summarize data relating to the ongoing progress of the interaction between PcG proteins and MYC oncogene in cancer, based on recent evidence. We report the associations with upstream and downstream effectors, and the networks that arise. Moreover, we describe the putative involvement of miRNAs as an intermediate link between the two afore-mentioned factors.

PcG protein interaction with MYC

PRC1 and MYC

Several years ago, Bmi1 was found to be a partner of Myc within the cell nucleus, leading to lymphomagenesis in a transgenic mice model, suggesting at that time that both factors are members of a transcription regulation complex [51, 52]. Sometime later, it was established that Bmi*1* overexpression was able to inhibit Myc-induced apoptosis in MEFs through the negative regulation of *Ink4a–Arf* in transgenic mice [53]. Since then, several studies have confirmed the positive association between *Bmi1* and *Myc* overexpression. Their interaction led to the formation of circuitries, and repression of TS genes in different subtypes of cancer, and it was also suggested that they may be involved in the generation of cancer stem cells (CSCs) [54–57].

BMI1 gene was found to be a direct transcriptional target of *c-MYC* in human diploid fibroblasts, and their co-regulation negatively regulated p16 mRNA and protein levels. However, physiological c-MYC levels did not affect p16, while hypoactive c-MYC altered p16 via BMI1. On the other hand, hyperactive c-MYC had a direct effect on *CDKN2A* (*p16*) promoter by direct binding to its promoter E-box [58]. These findings suggested that, in part, the combined action of BMI1 and M*YC* is necessary to modulate apoptosis through modification of cell cycle regulator expression.

Duss and colleagues [59] studied an estrogen-dependent transformation model of human mammary epithelial cells (HMECs) through lentiviral transduction of HMECs with estrogen receptor α (*ERα*), *Myc, Bmi1*, and *Tert* in mice. However, the *ERα/Bmi1/Myc/Tert*-transduced HMECs failed to survive in normal estrogen level conditions, while estrogen administration promoted tumorigenesis. Cells expressing Bmi1/ERα were biologically active and proliferating when exposed to estrogens. The addition of Myc accelerated tumor growth compared withthe *Bmi1/ERα* only cells. Given that only *Myc* cells failed to proliferate and that *Bmi1/ERα* proliferated slower than the *Bmi1/ERα/Myc* cells, it can be deduced that these factors are interconnected and increase each other's activity. In breast cancer cell lines, it was shown that the overexpressed

BMI1 positively correlated with Wnt1 and *c-MYC* expression. On the other hand, it negatively regulated the expression of Wnt inhibitors such as *Dickkof* (*DKK*), with the final result being the upregulation of Wnt target such as *c-MYC*. The specific TF participates in a positive feedback loop activating the transcription of *BMI1*. In turn, *BMI1* itself is a target of Wnt pathway, and *DKK1* expression downregulates both c-*MYC* and *BMI1*. *DKK1* and *BMI1* regulate the expression of each other in a negative feedback loop. Thus, the oncogenic activity of Wnt signaling pathway and *BMI1* is interconnected in a positive feedback loop via *c-MYC* [60].

Bmi1 is overexpressed in the granule cell lineage, which can give origin to medulloblastomas as observed in transgenic and knock out mouse models. Upregulated *Bmi1* corresponded to the expression of *Myc* and its upstream regulator *Lef1*, and a *Bmi1–Lef1–Myc* axis has been proposed [61]. *BMI1* was overexpressed in primary glioma samples and correlated with adverse prognosis and resistance to radio-chemotherapy. Interestingly, *BMI1* enhanced the transcriptional activity of the nuclear factor (*NF*)-*κB* TF by promoting its nuclear translocation and, most interestingly, *MYC* expression was induced among other NF-κB targets, suggesting a *BMI1–NF-κB–MYC* axis in glioma [62]. *MYCN* and *c-MYC* overexpression induced proliferation and tumor growth in human neuroblastoma samples and xenografts in a *Bmi1*-dependent pattern, exhibiting a positive correlation. Knockdown of *MycN/c-Myc* and *BMI1* led to decreased mitosis and karyorrhexis [63]. No correlation between BMI1 and MYCN/c-MYC in gliomas or medulloblastomas was observed, in contrast to the previous study [62], suggesting that the interaction is cell-type specific. Interestingly, in cases of MYCN amplification there was a positive correlation between BMI1 expression and MYCN. Nevertheless, in the non-amplificated MYCN cases, BMI1 expression correlated with c-MYC expression [63]. MycN was found able to protect neuroblastoma precursor cells from death stimuli through *p53* repression, whereas *Bmi1* overexpression induced the polyubiquitination and proteasomal degradation of p53 in a Ring1a/b-mediated p53 polyubiquitination as observed in a transgenic mice model of neuroblastoma [64].

PcG proteins promote ESCs and adult leukemic stem cell self-renewal maintenance by blocking cell fate decisions, contributing to oncogenesis. Akt phosphorylates Bmi1 at Ser^{316}, both in vitro and in vivo, impairing its chromatin-modifying function. Moreover, phosphorylated Bmi1 exhibits a suppressed growth-promoting potential, and effects on senescence and cellular transformation. Akt-mediated phosphorylation also promotes the dissociation from chromatin and the derepression of the *Ink4a–Arf* locus, while phosphorylated *Bmi1* loses its ability to cooperate with MYC in cellular transformation. Such interaction inhibits self-renewal of hematopoetic progenitor cells and inhibits ubiquitination of H2A. Thus, the PI3K–Akt pathway indirectly fine tunes cell growth by inhibiting *Bmi1* through Ser^{316} phosphorylation [65].

The FoxM1 TF, with known tumor-promoting properties in diverse cancer subtypes, is required for proper mitosis in MEFs, and its overexpression protects cells from oxidative stress-induced senescence. That action is achieved via upregulation of c-*Myc*, subsequent activation of its downstream target *Bmi1,* and final suppression of the *p19Arf–p53* pathway, resulting in MEFs protection from senescence [66]. These findings shed light into the molecular mechanisms of senescence and oncogenesis at the early transforming stages.

ID1 is an oncogene affecting cell proliferation, cell cycle progression, apoptosis, differentiation, invasion, and angiogenesis. ID1 exclusively affects PRC1 and regulates the expression of *MEL-18* and *BMI1* in human breast cancer cell lines. It enhances the E3 ligase activity of *RING1b* through the PI3K/AKT pathway, accumulating H2Aub and proteosomal degradation of geminin, which is a PRC1 target. ID1 induces downregulation of *MEL-18* via AKT ser^{473} phosphorylation and activation of the AKT signaling pathway, and *BMI1* activation. ID1 also enhances *c-MYC* transcription through inhibition of *MEL-18*. Small interfering RNA (siRNA)-mediated *MYC* downregulation abolishes *ID1*-mediated *BMI1* upregulation, while ID1 regulated BMI1 transcrition through c-MYC. It can also be suggested that the oncogenic function of *c-MYC* is attributed/enhanced by the oncogenic power of *ID1*. It can also be assumed that it affects CSCs biology via BMI1, c-MYC, and geminin, which regulates cell proliferation, differentiation, and genomic stability [67].

Other than Bmi1, PRC1 subunits were also observed to interact with Myc. In fact, Cbx7 is overexpressed, and exhibits tumor-initiating and disease-accelerating properties in contrast to other PcG proteins in transgenic mice. Cbx7 acts independently of Bmi1, repressing *Ink4a/Arf* TS locus in cooperation with Myc, promoting follicular lymphoma pathogenesis [68]. Members of the posterior sex combs proteins interact and regulate each other at the messenger RNA (mRNA) level. *MEL-18* downregulates *BMI1* in fibroblasts and accelerates the entry of cells into senescence by upregulating *p16* and increasing the growth inhibitory form of phosphorylated *Rb*. *C-MYC* binds to *BMI1* while *MEL-18* is not able to bind directly on *BMI1*. MEL-18 regulation on BMI1 is achieved by repressing c-MYC and by downregulating AKT. Interestingly, *c-MYC* overexpression rescued MEL-18 mediated repression of *BMI1* expression. MELl-18 expression was reduced in prostate cancer samples and breast cancer, and inversely correlated with BMI1 and c-MYC levels. MEL-18 forced expression

also attenuated cancer cell growth through G1 arrest via modulation of AKT signaling [69–73]. Therefore, the existence of a MEL-18–c-MYC–BMI1–p16–pRb pathway regulates cell senescence.

PRC2 and MYC

Less work has been done regarding PRC2 members and MYCc interaction, but the findings are most interesting. All three PRC2 core components were upregulated concomitantly to MYC in cell lines bearing 20q amplification, a chromosomal modification that occurs early in the malignant transformation process [74]. The interaction between EZH2 and c-MYC has been detected in the tumorigenic process as early as the CSC level. *EZH2* was overexpressed in primary glioblastoma CSC, promoting aberrant self-renewal by modulating the expression of direct downstream genes such as *c-MYC*. *C-MYC* was also capable of rescuing in part the effects of the EZH2 inhibitor 3-deazaneplanocin A (DZNep) treatment on CSC [75]. EZH2 was found to activate c-Myc in breast cancer cells through the ERα and the Wnt pathways, in a Wnt/b-catenin–EZH2–ERα–MYC axis [76].

In MLL-AF9 acute myeloid leukemia (AML), the genes of the MYC module were negatively enriched in EZH2-inactivated leukemic cells. These data suggest a functional link between gene expression programs that are under the control of PRC2 and MYC. These findings could also have therapeutic implications, since modulation of EZH2 might decrease the expression of MYC transcriptional targets at least in specific subtypes of AML [77].

H3K27me3 levels were decreased in prostatic intraepithelial neoplasia and prostatic adenocarcinoma and were independent from the high EZH2 levels. An Myc-expressing mouse model was studied in order to obtain better insight into decreased H3K27me3 levels. The findings supported the hypothesis that in vivo Myc overexpression results in a global decrease of H3K27me3 levels. These data suggested that Myc has the ability to influence epigenetic marks chromatin structure in cancer in a histone methyltransferase-independent pattern [78].

HOXB3 increases expression of DNMT3B, which is recruited and directly bound to *RASSF1A* promoter, repressing its expression via hypermethylation. DNMT3B recruitment is achieved through interactions with *EZH2* and *MYC*, which is also bound to RASSF1A promoter. In fact, MYC knockdown results in decreased EZH2 expression and *DNMT3B* recruitment. Epigenetic silencing of *RASSF1A* through HOXB3 induction of *DNMT3B* expression is commonly observed in lung adenocarcinomas and several other human cancer cell lines. Thus, a putative repressive mechanism involving the MYC association with EZH2 and DNMT3B on RASSF1A has been suggested [79]. Although this report shows that Myc is important for PRC2 recruitment, it is also true that Myc is not sufficient to recruit PcG proteins, as nearly 5 % of mouse ESC promoters bound by Myc are also bound by PcG proteins [80].

Recently, it has been demonstrated that Myc suppresses the PI3K/Akt pathway through transcriptional upregulation of its negative regulator the *PTEN* TS, initiating and maintaining gene repression. Furthermore, Ezh2 is activated by Myc-mediated suppression of Akt kinase activity, which in turn leads to Ezh2-mediated gene repression. Myc represses genes via Ezh2-induced H3K27me3, including Myc itself in rat fibroblasts, supporting a role of Ezh2 in Myc-mediated gene repression and autoregulation [81].

MYCN represses clusterin TS through direct interaction with a non-canonical E-box inducing bivalent epigenetic marks and recruitment of repressive enzymes such as histone deacetylases (HDACs) and PcG proteins. MYC recruits EZH2 to *clusterin* promoter inducing transcriptional silencing both in vivo and in vitro in neuroblastomas. Notably, although binding of MYCN was associated with active chromatin marks such as H3ac and H3K4me2, negative marks such as H3K9me3 and H3k27me3 were also observed immediately downstream of the E-box. In agreement with this observation, several chromatin remodeling factors associated with transcriptional repression such as *HDAC 1/2*, *BMI1*, *EZH2*, and *SUZ12* were detected around the E-box or downstream of the E-box sequence in the presence of MYCN. This 'bivalent' configuration is typical of repressed, developmentally regulated genes, which are poised to be activated by physiological stimuli [82].

Myc is also involved in stem cell biology, as both c-MYC and MYCN expression is required for ESCs and induced pluripotent stem cell (iPSC) self-renewal that could not be compensated by MYCL, highlighting the different biologic properties of each homolog in ESC biology. This regulatory function of MYC is achieved through the regulation of miRNAs involved in iPSC biology, modulation of cell cycle regulator expression, control of the euchromatin organization, and consequently the epigenetic state of iPSCs [48]. Accumulating evidence shows that PcG proteins and Myc are involved in stem cell biology, and induced pluripotency. Most recent findings establish that *c-MYC*, together with *KLF4*, are responsible for initiating the first transcriptional wave during somatic cell reprogramming [83]. Moreover, there is a Myc-centered network that acts independently of the other core TFs (Oct4, Sox2, Nanog) composing sub-signatures represented by the core TF network, the Myc network, and the Polycomb cluster [84]. Intriguingly, recent data revealed that inactivation of *Ezh2* in MEFs treated with Oct4, c-Myc, Klf4, and Sox2 TFs resulted in successful reprogramming, probably via Ezh1 recruitment on target genes and H3K27me retention [85].

ESCs can undergo rapid self-renewal and can differentiate into any cell type. That feature depends on TFs, including Oct4, Sox2, and Nanog, that form the core pluripotency network. This ESC-specific network interacts with both the Myc-based transcription network and a chromatin-modifying complex network including PRC2. Together these three networks occupy and regulate a large number of target genes essential for the self-renewal and differentiation of ESCs. Myc and PRC2 seem to be under the control of the Utf1 TF, which prevents PRC2 binding, while it blocks the Myc–Arf feedback loop, ensuring rapid proliferation of ESCs [86]. Thus, the three modules do not act separately but rather in conjunction, with Utf1 being the intermediate link.

Myc is involved in the transcriptional regulation of ESCs pluripotency network and in histone methylation mediated by PcG. Transcriptional activation of the entire PRC2, and not of individual core members, contributes to high H3K27me3 levels, therefore keeping bivalent genes silent. The final result of the Myc–PRC2 interaction was a maintained ESCs undifferentiated state, as established in double knockdown experiments, and the cell cycle progression maintaining self-renewal of ESCs [87].

Ben-Porath et al. [88] sought to determine whether the regulatory networks that characterize ESCs are also active in cancer. Four groups were defined, with 13 partially overlapping gene sets; the Polycomb targets and the Myc targets were among them. Their data suggested that poorly differentiated tumors display a molecular pattern similar to ESCs, and that cancer cells in such tumors are biologically closer to normal undifferentiated stem cells than are cells in well differentiated tumors.

C-MYC overexpression in human umbilical cord bloodmarrow stroma cells (hUCB-MSCs) induces the expression of PcG complex genes, and most PcG genes are downregulated after HDAC2 inhibition. However, the expression level of *PHC1*, *PHC2*, *RING1*, and *EZH2* are not downregulated after HDAC2 inhibition. This might indicate that these genes are not under the control of HDAC2 or c-MYC. A c-MYC regulatory feature might also be the regulation of PcG gene expression via HDAC2 control. As a result, cell proliferation and differentiation of adult stem cells is affected [89]. These studies on adult stem cells and iPSCs could act as guides to get further insight in the first steps of carcinogenesis.

Poly-MYCroRNAs: "ménage a trois"

miRNAs are a class of non-coding RNAs with regulatory function of gene expression. They are involved in the regulation of physiological processes, in pluripotency, in reprogramming and in several different diseases including cancer [90–92]. Their function is achieved through modulation of cell signaling, differentiation, proliferation, organogenesis, development, and apoptosis. miRNA genes are distributed in all human chromosomes except for the Y chromosome. Half of them are found in clusters and they are transcribed as polycystronic primary transcripts [93, 94].

More than 2,000 human mature miRNA sequences are included in miRBase release 19 accounting for the 1–2 % of the human genome, with the ability to control the activity of nearly 50 % of all coding genes [95].

miRNA biogenesis is a complex multistep process involving several proteins. Canonical and alternative miRNA biogenesis pathways have been described, and our knowledge in their biogenesis is expanding every day. In the canonical pathway, the pri-miRNAs generated by RNA Pol II are cleaved in the nucleus by the RNase III DROSHA in conjunction with the DGCR8 protein. These new pre-miRNAs are exported to the cytoplasm by Exportin-5 protein, where they are further cleaved by DICER, reaching their final ~22 nt length. The resulting double-stranded small RNA is loaded onto the Argonaute (Ago) proteins, forming the effector complex RNA-induced silencing complex (RISC). One RNA strand remains attached to the Ago as a mature miRNA while the other strand is degraded [94, 96]. Alternative miRNA biogenesis pathways that bypass DICER and/or DROSHA/DGCR8 have also been identified [97, 98].

miRNAs are able to regulate their target gene expression by base pairing with the 3′ untranslated region (UTR) of the target mRNA, although there is evidence supporting that targets can be located in the 5′UTR or in coding regions of genes. miRNA target sites exhibit perfect matching between the nucleotides 2–7 of a single miRNA and the mRNA, whereas mutations in the target genes might lead to novel target sites [99]. Through that interaction, a single miRNA is capable of repressing tens to hundreds of targets. Several mechanisms of target regulation have been proposed, including stimulation of translation, endonucleolytic cleavage, deadenylation and degradation of the mRNA, inhibition of translation initiation, and inhibition after translational initiation. All these mechanisms finally consolidate gene silencing [100].

miRNAs possess regulatory functions of fundamental signaling pathways such as Wnt, NOTCH, Hedgehog, RAS, and MAPK/PI3K/AKT [101, 102]. MiRNAs have the ability to act either as TS or as oncogenes depending on the type of genetic or epigenetic abnormality present. They have multiple roles in tumor genesis and progression, as they are capable of modulating oncogenic, TS pathways, and metastasis pathways, including c-MYC, p53, RAS, and BCR/ABL, the TWIST1–miR10b–HOXD10 pathway. Nevertheless, their expression can be regulated by other oncogenes or TS [102, 103]. They are involved in almost

all types of human cancer, and they can classify human cancer according to the differentiation state and developmental lineage of the cancer [104]. Their expression is significantly associated with major cancer outcomes, while they can be used as biomarkers for disease progression and response to treatment [104–108]. A more detailed description of miRNA biogenesis, function, and regulation is beyond the scope of the present manuscript.

miRNAs act at the transcriptional and post-transcriptional level and they are regulated by TFs, forming complex regulatory networks affecting each other's expression. Both TFs and miRNAs form feedback and feedforward loops through which their target gene expression is regulated [109]. Thus, miRNAs are closely associated with MYC. For example *miR-150* is under the negative control of *MLL-fusion/Myc/Lin28* axis in MLL-rearranged AML [110]. Moreover, several other TS miRNAs are downregulated by MYC in lymphoma cells by direct binding to their promoters, promoting lymphomagenesis [111]. *MiR-22* and *MYC* form feedforward loops in cells that exit a quiescent state and enter a proliferative state characteristic of malignant transformation [112]. MYC has the ability to suppress proline oxidase and proline metabolism in cancer cells through mir-23b* upregulation, affecting in that way another hallmark of cancer [113].

However, miRNAs also interact with the PcG members. EZH2 is under the control of several different miRNAs affecting H3K27me3 levels of target genes, whereas EZH2 itself activates or represses other miRNAs [114]. Specific miRNAs have the ability to promote myogenesis and terminal differentiation in mouse myoblast cell lines. Among them, the upregulated expression of *miR-26a* was required during terminal differentiation in order to induce rapid and efficient silence of Ezh2, which is a negative regulator of myogenesis Ezh2 [115].

The interaction between MYC-miRNAs and PcG proteins is quite complex, as these factors compose sophisticated modulatory networks. *MiR-29* has dual oncogenic or TS function depending on the cellular context and it seems that acts as TS in MYC-associated lymphomas. *MiR-29* is MYC-repressed with the cooperation of HDAC3 and EZH2 in MYC-associated lymphomas cell lines and in primary samples. Interestingly, MYC recruits EZH2 and SUZ12 at *miR-29* promoter, promoting its epigenetic silencing. MYC depletion leads to decreased recruitment of RNA pol II, HDAC3 levels, and EZH2, promoting increased histone acetylation and decreased H3K27me3, respectively. An important finding of this study is the identification of the *MYC–miR-26a–EZH2–miR-494* positive feedback loop that sustains MYC activity and consequent *miR-29* repression. MYC leads to EZH2 upregulation through *miR-26a* repression and in turn *EZH2* suppresses *miR-494*, which targets *MYC*. That loop confers the *MYC* oncogene persistent high protein levels and further repression of *miR-29*. This study sheds light on a putative mechanism of *EZH2* activation and contribution to tumor aggressive transformation [116].

Ezh2 can epigenetically repress miRNAs, enhancing the expression of *Bmi1* and *Ring2*, promoting H2AK119ub in advanced prostate cancer. Therefore, miRNAs also act as the intermediate link for the coordinated function of PRC1 and PRC2 in cancer [117]. The interaction between miRNAs and PcG proteins could indirectly affect the fate of the hematopoietic progenitor. In fact, an *miR-223/PcG* axis regulates the *NFI-A* gene affecting hematopoietic cell lineage determination [118]. *MiR-18a* and *miR-19a* were upregulated and transctivated by *MycN* in neuroblastoma and they negatively regulated *ERα1* expression [119].

MYC and *EZH2* were overexpressed and positively correlated in primary samples and in a mouse model of prostatic intraepithelial neoplasia. However, as *MYC* regulates the expression of *miR-26a/b*, and *miR-26a/b* targets *EZH2* in prostate cancer cells only and not in breast cancer or AML, it could be suggested that the *MYC–miR-26a/b–EZH2* interaction presents a tissue-specific pattern. Final result of the deregulated axis is the maintained proliferative capacity of

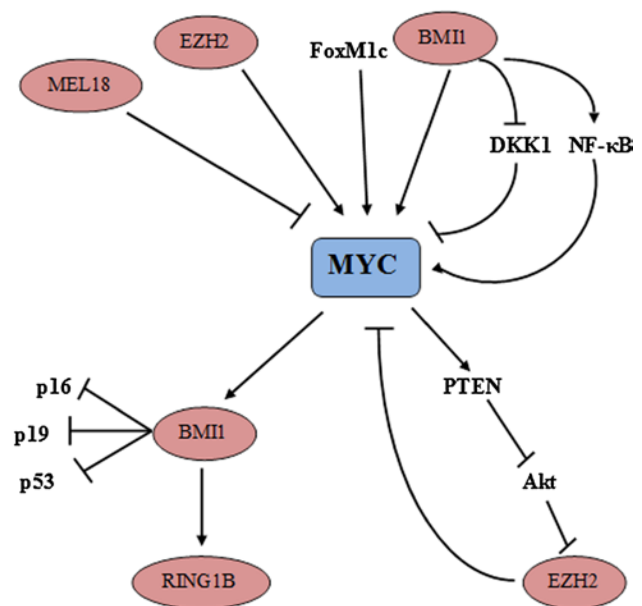

Fig. 1 Direct upstream regulators and downstream effectors of MYC oncogene are shown. MYC expression is directly regulated by other transcription factors (FoxM1c) and by different polycomb proteins (EZH2, BMI1, Mel-18). BMI1 also regulates MYC via other transcription factors (nuclear factor [NF]-κB) or via Wnt pathway inhibitors such as Dickkof (DKK1). EZH2 is able to activate c-MYC in a Wnt/b-catenin–EZH2–ERα–MYC axis. MYC represses genes via EZH2, including MYC itself, forming autoregulatory loops with EZH2. MYC controls the polycomb repressive complex (PRC)-1 member RING1B through regulation of its direct downstream regulator BMI1. In turn, BMI1 affects the expression of cell cycle regulators. For a more detailed description see the text

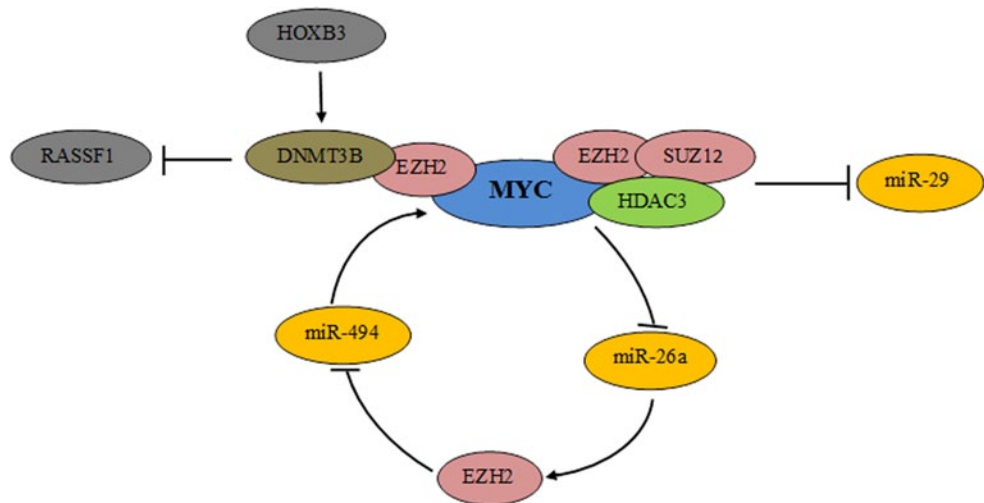

Fig. 2 MYC interacts with the histone methyltransferase EZH2 and recruits the DNA methyltransferase DNMT3B, expression of which is enhanced by HOXB3, on the promoter of RASSF1 tumor suppressor repressing its expression. MYC also binds to EZH2/SUZ12 and in cooperation with the histone deacetylase HDAC3 promote silencing of miR-29. MYC is a part of a MYC/miR-26a/EZH2/miR-494 regulatory loop enhancing MYC expression

cancer cells. Therefore, EZH2 can also represent an early prostate cancer contributor and a driver of disease progression, and its expression could be enhanced by MYC via two mechanisms (directly or through miR-26a/b) [120].

MYC has the ability to alter PRC2 by interacting with the TS *miR-26a* or by targeting E2F1 proapoptotic protein [121]. *miR-26a* is downregulated in MYC-induced lymphoma, whereas its direct negative target *EZH2* is overexpressed [122]. That interaction might result in MYC-induced EZH2 expression through downregulation of its target miRNA. In AML samples, MYC directly enhanced *EZH2* transcription, while it also repressed *miR-26a* transcription [123].

miR-26a was also downregulated in nasopharyngeal (NPC) primary samples and cell lines promoting tumor growth, while negatively correlated with EZH2 levels. *miR-26a* directly targeted *EZH2* in NPC cells and it was demonstrated that ectopic EZH2 expression rescued miR-26a cell growth inhibition and cell cycle arrest. The effect of forced miR-26a expression on tumor growth inhibition was in part mediated by downregulation of c-MYC together with other cell-cycle regulators [124].

Treatment options

MYC seemed to represent an attractive therapeutic target as it fulfilled the required criteria for optimal therapeutic efficacy [125]. However, the enthusiasm has switched to skepticism, and a search for alternative approaches for several reasons [126]. Drugs able to inhibit c-MYC/MAX dimerization, and to decrease global H3K9ac and increase H3K9me2 levels, such as Omomyc, have been developed [127, 128]. Other alternative approaches for inhibiting *MYC* expression have been developed, such as inhibition of the bromodomain and extraterminal (BET) subfamily of human bromodomain proteins, or the use of antogomirs and miRNA mimics to inhibit or activate oncogenic and TS miRNAs, respectively, that are associated with Myc function [129–131].

Similarly, pharmacologic inhibition of EZH2-activating mutations in lymphoma has been reported. DZNep is the most studied EZH2 inhibitor in different cancer subtypes with a targeting potential that reaches the CSC compartment [132–135].

However, there is no evidence of how inhibition of MYC or PRC could affect each other's expression. Moreover, none of the currently known drugs have been reported to affect both MYC and PcG and modulate their interaction. Recently, it was described that genistein, a botanical isoflavone enriched in soybean products, induces the expression of $p21^{waf1}$ and $p16^{ink4a}$ and downregulates both Bmi1 and c-Myc [136]. C-Myc itself was determined to be an HDAC inhibitor target, which also possesses the ability to indirectly suppress Bmi1 and EZH2 transcription in breast cancer cell lines, leading to reactivation of PcG target genes [137]. The effect of HDAC inhibitors and of genistein on Myc in cancer biology should be further explored.

Conclusions

It is widely accepted that PcG proteins and MYC are involved in several physiogical processes, induced pluripotency, and cancer, acting independently or in conjunction. PcG proteins regulate chromatin organization, whereas MYC might also control global chromatin organization through regulation of target gene transcription. PcG proteins also exhibit gene-expression regulator characteristics and might act as fine tuners of MYC-induced changes in cell biology. Although MYC binding to its targets correlates with specific epigenetic changes, it is unclear whether

MYC establishes these marks or is recruited to target promoters as a consequence of chromatin modifications. MYC and PcG protein interactions are involved in the regulation of most important signaling pathways (Fig. 1). MYC might promote the transcription of TS, which in turn suppress signaling transduction and finally enhances its oncogenic potential via PcG target gene repression. Moreover, MYC, PcG proteins, and miRNAs form complex networks, feed-forward and feedback loops regulating gene expression and controlling each other's expression (Fig. 2). PcG proteins have been shown to be able to substitute MYC in induced pluripotency. However, given that the function of these genes is not completely clear and understood, we must be prudent before using them widely. Accumulating data continuously describe new complex circuitries in which PcG proteins and MYC are deregulated in cancer, with prospective therapeutic implications against both blood and solid cancers.

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Conflict of interest None.

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