Published in final edited form as:

Trends Cancer. 2018 December ; 4(12): 810–822. doi:10.1016/j.trecan.2018.10.003.

The MYC enhancer-ome: Long-range transcriptional regulation of MYC

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Abstract

MYC is one of the most important oncogenes in cancer. Indeed, *MYC* is upregulated in 50–60% of all tumors. *MYC* overexpression can be achieved through a variety of mechanisms, including gene duplications, chromosomal translocations or somatic mutations leading to increased *MYC* stability. However, recent studies have identified numerous tissue-specific non-coding enhancers of *MYC* that play major roles in cancer, highlighting long-range transcriptional regulation of *MYC* as a critical novel mechanism leading to *MYC* hyperactivation and as a potential target for new therapeutic strategies in the near future. Here, we summarize the regions and mechanisms involved in the long-range transcriptional regulation of *MYC*, underscoring the relevance of *MYC* enhancers both in normal physiological development and in *MYC*-driven cancer initiation and progression.

Keywords

MYC; Enhancer; long-range transcriptional regulation; cancer; SNP

1. MYC overexpression in cancer

The *MYC* oncogene encodes a basic helix-loop-helix leucine-zipper (bHLH-LZ) transcription factor which heterodimerizes with MAX in order to transactivate its target genes. The transcriptional program controlled by *MYC* drives a wide variety of cellular processes involved in normal cellular proliferation and in malignant transformation, including ribosome biogenesis, nucleotide biosynthesis and metabolism [1]. Therefore, its deregulation can have serious consequences. Indeed, *MYC* has been linked to cancer development since its original discovery as a target of retrovirally mediated tumorigenesis [2, 3]. Numerous subsequent studies collectively revealed *MYC* as one of the most

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The authors declare no competing financial interests.

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important oncogenes in human cancer. Specifically, amplification of *MYC* paralogs occurs in ~30% of all tumors [4], whereas upregulation of *MYC* expression has been observed in 50–60% of all cancers and directly contributes to malignant transformation through its pathogenic roles in tumor initiation, progression and maintenance [5]. *MYC* overexpression has been extensively studied in literature. The mechanisms behind this increase in *MYC* levels typically involve gene amplifications, chromosomal translocations, epigenetic changes, transcriptional regulation, somatic mutations, protein stabilization or activation of upstream signal transduction pathways [6].

However, the development of Next Generation Sequencing techniques in the last decade prompted a revolution in cancer genomics and led to the discovery that most of the cancer-associated mutations and copy number alterations occur in non-coding regions [7]. In turn, this led to the identification of enhancer malfunction as an important novel mechanism implicated in cancer and, specifically, as a major mechanism contributing to *MYC* deregulation [8].

Enhancers are non-coding and long-range cis-regulatory elements that control gene transcription through three-dimensional physical interaction with gene promoters, independently of their position and orientation with respect to the transcription start site [9]. Seminal work on the regulation of the globin enhancer/locus control region/promoter in a spatiotemporal specific manner laid the foundations for the enhancer biology field [10, 11]. In the last years, methods such as chromatin immunoprecipitation sequencing (ChIP-seq), DNase I hypersensitivity sequencing (DNase-seq), Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq) and, in particular, the development of Chromosome Conformation Capture technologies (3C, 4C, 5C, ChIA-PET, Capture-C or Hi-C, among others) [12] have been critical for the discovery and dissection of enhancer regions, and have collectively revealed that these regulatory elements are characterized by: (i) the presence of histone modifications such as mono-(ATAC-seq) and, in particular, the development of Chromosome Conformation Capture technologies (3C, 4C, 5C, ChIA-PET, Capture-or Hi-C, among others) [12] have been critical for the discovery and dissection of enhancer regions, and have collectively revealed that these regulatory elements are characterized by: (i) the presence of histone modifications such as mono methylation of histone H3 at lysine 4 (H3K4me1) and acetylation of histone H3 at lysine 27 (H3K27ac); (ii) the binding of proteins involved in the establishment and maintenance of chromatin loopings, such as Cohesin, Mediator and CTCF; (iii) the binding of key transcription factors that control enhancer activity; and (iv) overall increased chromatin accessibility [13–15]. In addition, Hi-C experiments have revealed that the genome is organized in regions, referred to as topologically associated domains (TADs), and both cohesion and CTCF play critical roles in the establishment and maintenance of TADs [16, 17]. Importantly, most of the chromatin interactions happen intra-TADs, with much fewer interactions between different TADs [16, 17]. Moreover, most of the putative cis-regulatory elements remain normally inaccessible due to nucleosome occupancy, however, a subset of sequence-specific transcription factors can act as pioneer factors and overcome this occlusion in order to bind to their specific sequences [18]. Pioneer factors make the underlying DNA more accessible to transcriptional machinery and this, in turn, allows the recruitment of the Mediator complex, which facilitates enhancer interaction with the basal transcription machinery and

RNA polymerase II at promoters in a gene-specific manner [19]. Finally, these regulatory regions can also be found in large clusters of individual enhancers located in close genomic proximity, defined as super-enhancers, which typically control the expression of major developmental factors. In addition, super-enhancers show a higher level of transcription factors bound to these regions, resulting in the increased expression of their target genes [20].

Notably, *MYC* is located inside a ~3-megabase area devoid of protein-coding genes on chromosome 8q24 that corresponds to a single TAD, with several subTADs defined by CTCF binding sites, thus favoring chromatin interactions inside the *MYC* TAD [21, 22]. Interestingly, it has long been known that this ~3-megabase-long non-coding region contains a great number of SNPs associated with higher risk of developing certain types of cancer, although not all of these SNPs have been functionally validated to affect *MYC* levels [23]. In this context, numerous studies have recently identified a multitude of tissue-specific enhancers and super-enhancers of *MYC* in this region, and these regulatory regions have been shown to play a critical role fine-tuning *MYC* expression both during normal development and transformation. Indeed, tissue-specific *MYC* enhancers have been found to be the target of recurrent duplications in cancer, to be enriched in cancer-associated SNPs and/or to be hijacked and aberrantly activated by additional cancer types. Overall, these findings underscore long-range regulation of *MYC* as a novel mechanism leading to *MYC* hyperactivation in cancer and as the current focus of intense research efforts with the hope of eventually finding ways to specifically target *MYC* (a prototypical “undruggable” target) in subsets of cancer cells, while sparing normal cells.

Here, we will review the full enhancer landscape of *MYC* (Figure 1), or *MYC* “enhancer-ome”, and summarize the regions and mechanisms involved in the long-range transcriptional regulation of *MYC* in different cancer types (Table 1). Of note, we will focus on the enhancer regions of *MYC* identified in the 8q24 locus, not on the translocations that juxtapose *MYC* with other enhancer regions such as the IgH super-enhancer in multiple myeloma [20] and Burkitt lymphoma [24], the T-ALL cases that juxtapose *MYC* with the TCR regulatory region [25] or other similar rearrangements. For more comprehensive reviews on *MYC* in cancer or on the role of enhancer regions in tumorigenesis, see references [1, 8, 26–29].

2. Long-range regulation of *MYC* in hematological malignancies

• B-Cell malignancies

Chronic Lymphocytic Leukemia (CLL) is the most common leukemia (~40% of all adult leukemias) and it is characterized by the accumulation of a clonal population of double-positive CD5⁺ and CD19⁺ B-lymphocytes in blood, bone marrow and secondary lymphoid organs [30]. In CLL, two different single nucleotide polymorphisms (SNPs), namely the rs2456449 (chr8:127,180,736) and rs2466024 (chr8:127,175,774) variants, were originally identified as predisposing factors for CLL. Interestingly, these SNPs are located in the long region devoid of protein coding genes 500Kb upstream of *MYC*. Although initial studies could not demonstrate an effect on *MYC* expression [31], subsequent reports identified two distinct enhancers of *MYC* in this region that together form part of a super-enhancer.

Importantly, these enhancers were found to be positively regulated by NOTCH1 and to be recurrently duplicated in ~3–4% of primary CLL cases [32]. In addition, this super-enhancer also shows H3K27ac marks and interaction with the *MYC* promoter in Small Lymphocytic Lymphoma and Mantle Cell Lymphoma [33–35]. Thus, NOTCH1 regulates *MYC* expression in B-Cells through this enhancer, named B-NDME (for **B**-Cell **N**otch-**D**ependent **MYC** **E**nhancer). Indeed, CRISPR/Cas9-induced deletion experiments revealed that B-NDME is required for *MYC* expression and growth of NOTCH1-dependent Mantle Cell Lymphoma cell lines *in vitro* [34].

• T-Cell Acute Lymphoblastic Leukemia

T-Cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive hematologic malignancy resulting from the transformation of immature bone marrow progenitors. T-ALL accounts for 10 to 15% of pediatric and 25% of adult acute leukemia cases [36]. Importantly, T-ALL is primarily a NOTCH1 driven disease since more than 60% of the cases harbor activating mutations in NOTCH1, and NOTCH1 itself had been long-known to drive *MYC* activation through unclear mechanisms [37]. In this context, two independent studies recently identified a T-Cell specific and NOTCH1-driven enhancer of *MYC* (N-Me, for **N**otch1-driven **Myc** **E**nhancer), located 1.4Mb downstream of the *MYC* locus [38, 39]. Importantly, N-Me is recurrently duplicated (5%) in T-ALL patients, highlighting its relevance in the pathology of T-ALL [38]. Moreover, mice with Conditional and inducible deletion of N-Me revealed that this cell-type specific enhancer is critically required for normal T-Cell development, as N-Me deleted mice show a marked decrease in thymic cellularity, thymocyte proliferation and differentiation [38]. In addition, germinal deletion of N-Me resulted in reduced penetrance or complete abrogation of NOTCH1-driven leukemia development *in vivo*, whereas secondary deletion of N-Me in already established leukemias impaired leukemia propagation and also led to drastically reduced leukemia-initiating cell activity, consistent with the critical role of *MYC* in leukemic stem cells [38, 40]. Furthermore, N-Me has been found to require not only NOTCH1 but also β -Catenin and RUNX1 to induce *MYC* transcription in T-Cells. Indeed, β -Catenin also physically binds to N-Me, and germinal deletion of β -Catenin abrogates leukemia development in a NOTCH1-driven model of T-ALL [41]. Similarly, RUNX1 binds to N-Me, and secondary deletion of RUNX1 in already established T-ALLs abrogated the binding of NOTCH1 and resulted in impaired transcription of *MYC* [42]. In addition, it has been recently described that NUP214-ABL1/TLX1-positive T-ALL cases are characterized by a STAT5 gene signature in which both STAT5 and TLX1 cooperatively bind the N-Me enhancer to promote *MYC* expression [43]. Overall, these results highlight the role of N-Me as a major enhancer node driving T-cell proliferation and transformation through the integration of input signals from NOTCH1 and additional transcription factors.

• Acute Myeloid Leukemia

Acute Myeloid Leukemia (AML) is a heterogeneous disease that accounts for ~80% of the acute leukemias diagnosed in adults, being thus the most common acute leukemia in this group [44]. In AML, several studies identified areas in the 8q24 region that were recurrently duplicated (3%) in patients [45, 46]. A following report identified that the SWI/SNF complex is essential for the regulation of *MYC* transcription in AML cells by occupying a

cell-type specific super-enhancer. This super-enhancer region, located 1.7Mb downstream of and originally described to be composed of 5 distinct smaller enhancers, is able to physically loop with the *MYC* promoter and overlaps with the region initially found as the target of recurrent focal amplifications in AML [47]. A very recent study revealed that this super-enhancer region, renamed as BENC (for **B**lood **E**Nhancer **C**luster), is actually composed of up to 9 different enhancer peaks [48]. Notably, analyses of mice harboring specific deletions in single or multiple of these BENC-enhancer peaks uncovered additional and combinatorial activities for these independent enhancer elements. Importantly, this study demonstrated that this region is not only critically required for the development of MLL-AF9-driven AMLs *in vivo*, but it is also required for the self-renewal, proliferation and differentiation potential of Hematopoietic Stem Cells (HSCs). Specifically, BENC was shown to be important for the differentiation of both myeloid and B-Cell lineages, which raises the possibility that duplications of this region might also be found in B-Cell malignancies in the future [48]. In addition, the BENC enhancer and two other proximal enhancers (ME1 and ME2) located 0.18 and 0.5Mb downstream of the *MYC* transcription start site have been shown to play a prominent role in AML cases with inversion of chromosome 16 [49]. These AML cases are characterized by the expression of the fusion oncoprotein CBF β -SMMHC. Importantly, CBF β -SMMHC can bind to the transcription factor RUNX1, thus preventing RUNX1 binding to the ME1, ME2 and BENC enhancers. Notably, RUNX1 binding to these enhancer regions leads to the transcriptional repression of *MYC* (contrary to its effects on T-ALL), thus, the fusion oncoprotein CBF β -SMMHC sequesters RUNX1 resulting in the net transcriptional upregulation of *MYC*. Indeed, CRISPR-Cas9-mediated deletion of either BENC, ME1 or ME2 results in decreased *MYC* expression and impaired viability of inv(16) AML cell lines *in vitro* [49]. Finally, it has been suggested that there might be a cross-talk between the N-Me T-ALL enhancer and the BENC AML enhancer since T-ALL cell lines show H3K27ac marks in the BENC enhancer as well, and T-ALL cells selected to be resistant to NOTCH1 inhibition seem to switch from the N-Me enhancer to the BENC enhancer in order to maintain *MYC* expression [39].

3. Long-range regulation of *MYC* in solid tumors

• Prostate Cancer

A variety of genomic studies have associated multiple different SNPs and regulatory regions located in chromosome 8q24 with high risk of prostate cancer [50–57], the most prevalent cancer in males [58]. 8q24 contains at least three independent risk regions for prostate cancer, with several disease-associated SNPs described in each of them: region 1 (chr8:127,458,528–127,537,878); region 2 (chr8:127,062,671–127,162,671); and region 3 (chr8:127,389,527–127,458,527) [23, 51, 53–55, 59]. Importantly, it has been demonstrated that these regulatory regions physically interact with *MYC* in a tissue-specific manner [50]. Moreover, while some of the described SNPs are associated with prostate-specific cancer predisposition [51, 54, 55], the SNP rs6983267 (chr8:127,401,060; region 3) confers increased risk not only for prostate, but also for colorectal cancer [60]. Indeed, this region 3 encompassing the SNP rs6983267 is a bona-fide enhancer region that interacts with the *MYC* promoter and shows differential enrichment of H3K4me1, p300 and RNAPolII in colorectal cancer [61]. In addition, transgenic mice harboring the rs6983267 risk variant

showed prostate-specific enhancer properties for this SNP, leading to an increase in beta-galactosidase expression in the prostate which paralleled the levels of *MYC* [57]. Importantly, a very recent study identified recurrent duplication peaks ~700Kb and ~300Kb upstream of *MYC* in ~25% of metastatic castration-resistant prostate cancer samples [62]. The farther region included three different long non-coding RNAs together with region 2, whereas the nearer region included additional non-coding genes, as well as region 3 (with the SNP rs6983267) and region 1 (with the SNP rs1447295). In addition, an independent study also reported tandem duplications in castration-resistant prostate cancer samples in a ~500Kb upstream region encompassing the three risk regions (Chr8: 127.0–127.6) [63]. Overall, these results demonstrate the importance of the non-coding enhancer landscape of *MYC* in prostate cancer development.

• Breast Cancer

Breast Cancer is the most common cancer in women and the second main cause of cancer death in female patients after lung cancer [58]. High susceptibility to develop breast cancer has been associated with the rs13281615 SNP upstream of *MYC* (chr8:127,343,372) [52], and a broader region encompassing this SNP (chr8:127,337,755–127,497,755) was shown to physically interact with the *MYC* promoter in MCF-7 breast cancer cells, but not in a normal breast epithelial cell line [50]. Consistently, deletion in mice of the complete 538Kb region upstream of *Myc*, which encompasses several tissue-specific enhancers of *Myc* in prostate, breast and colon cancer, is dispensable for normal breast development and tissue homeostasis [64]. However, specific deletion of this super-enhancer region in mice confers partial resistance to dimethylbenz[a]anthracene/medroxyprogesterone (DMBA/MPA)-induced mammary tumors, pointing towards a critical role for this enhancer region in breast cancer generation as compared to normal breast development [64]. In addition, a recent study uncovered that the promoter of *PVT1*, which encodes an oncogenic lncRNA downstream of *MYC*, can actually compete with the *MYC* promoter for engagement with four intragenic enhancers in the *PVT1* locus in breast cancer cells [65]. Thus, CRISPR/Cas9-induced repression of *PVT1* drives increased *MYC* expression by facilitating the looping of the *MYC* promoter to these enhancer regions and results in increased proliferation. In addition, these enhancers in the *PVT1* sequence have also been shown to promote *MYC* expression in other tissues [66, 67]. Moreover, recurrent mutations in the *PVT1* promoter have been identified in breast cancer and lymphoma, highlighting the relevance of this mechanism of enhancer competition [65].

• Colorectal Cancer

Colorectal Cancer (CRC) results from the malignant transformation of epithelial cells in the large intestine. It is a major health problem and a leading cause of cancer-related mortality [58]. In this type of cancer, there are tissue-specific risk alleles located 335Kb upstream of *MYC* [50, 60, 61, 68]. This colon cancer risk region, which is the same as prostate cancer region 3 (chr8:127,389,527–127,458,527), has been shown to physically loop and interact with the *MYC* promoter in LS174T colon cancer cells *in vitro* [50]. Furthermore, the association of rs6983267 (chr8:127,401,060) with both colon cancer and prostate cancer underscores a common biological underlying mechanism for cancer risk at this 8q24 region [60, 61]. Thus, this SNP lies within a consensus binding site for the transcription factor

complex β -catenin/TCF4, and both TCF4 and β -catenin show enhanced binding to the CRC-associated G allele over the T variant. Interestingly, in TCF7L2 has been recently described to associate with the rs6983267 SNP G allele, promoting *MYC* activation and CRC cell proliferation [72]. Moreover, deletion of a ~1.7Kb region in mice containing this SNP leads to moderately reduced *MYC* expression levels in intestinal crypts, without any other apparent effects in normal healthy tissue but, most importantly, drastically reduces colon cancer development in the APC^{min} mouse model [64, 73]. Therefore, this regulatory region functions as a tumor-specific enhancer that is largely dispensable for normal intestinal function but required for tumorigenesis. This result is in sharp contrast with the effects of the N-Me enhancer in T-cells, where it is required both for normal T-cell development and for oncogenic transformation [38].

• Urinary Bladder Cancer

Urinary Bladder Cancer (UBC) is the sixth most common type of cancer in the United States, with approximately 79,000 new cases and 16,000 deaths from the disease in 2017 [58]. It has been long-known that the allele in SNP rs9642880 (chr8:127,705,823) was associated with the development of this type of cancer [74]. This SNP, located in a 1.6Kb insulator element ~30Kb upstream of *MYC*, confers a ~1.5x risk of developing UBC in homozygous carriers for the T allele, as compared to noncarriers [74]. However, mice lacking the full 538Kb region encompassing this SNP do not show decreased *Myc* levels in normal bladder, nor differences in bladder cancer development using the N-Butyl-N(4-hydroxybutyl)nitrosamine (BBN) chemically-induced carcinogenesis [64]. One possibility to interpret these seemingly contradicting results would be that this enhancer is dispensable for normal development of bladder, similar to its being dispensable for mammary gland development [64], and that *MYC* levels both in normal development and in bladder cancer are normally maintained by other enhancers/mechanisms, whereas only the presence of the T allele would result in specific hyperactivation of *MYC* in bladder cancer. Further gain-of-function studies using mouse models to specifically model the T allele would be needed to address this question.

• Medulloblastoma

Medulloblastoma is a highly malignant pediatric brain tumor classified into four biologically and clinically distinct molecular subgroups where *MYC* is the driver gene of group 3. Importantly, group 3 shows the worst overall outcome, with ~45% of metastasis already at diagnosis and 5-year overall survival of ~50% [75]. Many of these cases harbor focal duplications of the *MYC* gene, however, a 2016 report identified a novel enhancer 90Kb upstream of *MYC* that is a hotspot for focal amplifications in group 3 and was inferred to regulate *MYC* [76]. In addition, functional validation experiments in zebrafish *in vivo* demonstrated that this enhancer is highly specific to the developing hindbrain and/or medulloblastoma, underscoring the involvement of this non-coding region in the pathology of this tumor type [76].

• Merkel Cell Carcinoma

Merkel Cell Carcinoma (MCC) is a rare and aggressive neuroendocrine cutaneous malignancy associated with a poor prognosis [77]. A putative super-enhancer region

(Chr8:127,687,755–127,987,754) in *MYC* overexpressing MCC cell lines and primary MCC tumors was identified by strong enrichment in BRD4 binding and high levels of H3K27ac mark, together with low levels of H3k4me3. Interestingly, depletion of BRD4 occupancy at this regulatory region upstream of *MYC* results in a decrease of *MYC* expression and a marked inhibition of tumor growth *in vitro* and *in vivo*, suggesting that this super-enhancer might be involved in the regulation of *MYC* expression and tumor progression in MCC [78].

• Pancreatic Cancer

Pancreatic cancer is one of the most malignant and chemotherapy-resistant tumors and the fourth leading cause of cancer-associated deaths in the United States [58, 79]. Experiments done in the PANC-1 pancreatic adenocarcinoma cell line identified a super-enhancer with high levels of H3K27ac in close proximity to the 3' region of *MYC* [20]. Interestingly, this super-enhancer is not present in normal healthy pancreatic cells, suggesting that pancreatic cancer cells might acquire this super-enhancer *de novo*. In addition, a recent study identified frequent amplifications in the 8q24 region (~30% of cases), encompassing both the *MYC* gene and this pancreatic cancer-specific super-enhancer [80], suggesting a potential role in the pathogenesis of this disease. However, whether some patients might show amplifications of the super-enhancer alone is still unknown, and further studies are required to address its potential role in pancreatic cancer *in vivo*.

• Clear Cell Renal Cell Carcinoma

Kidney cancer is the eighth most prevalent cancer diagnosed each year in the United States, with an estimated 64,000 new cases in 2017 [58]. In 2013, GWAS studies identified the rs35252396 variant (chr8:127,877,125), a two base-pair substitution specifically associated with renal cancer susceptibility, located 136Kb downstream of *MYC* [81]. ccRCC is characterized by the loss of von Hippel-Lindau (VHL) gene and concomitant hyperactivation of HIF1 α . Importantly, Capture-C experiments revealed that this SNP resides inside an enhancer (HRE for **H**ypoxia **R**esponsive **E**lement) that interacts both with *MYC* and *PVT1* and is necessary for HIF1 α -mediated transactivation of both genes as demonstrated by CRISPR/Cas9-mediated deletion experiments. In this context, this risk-associated SNP polymorphism increases the chromatin accessibility and HIF1 α binding to this enhancer, resulting in increased levels of *MYC*, which is consistent with its association with higher ccRCC risk [82].

• Neuroblastoma

Neuroblastoma is a neuroendocrine tumor of the peripheral sympathetic nervous system (PSNS) that occurs very early during childhood (90% of patients are <10 years of age). Indeed, it is the most common extracranial tumor occurring in children (25–50 cases per million) and the age at diagnosis is highly prognostic, with much better survival rates in patients diagnosed <18-months. The main genetic alterations in this disease involve amplifications of *MYCN* or *ALK*, as well as *LIN28B* polymorphisms [83]. In this context, even if *MYCN* is one of the main drivers in this disease, the coding sequence of the *MYC* gene is generally not amplified in human neuroblastoma patients [84]. However, a very recent study demonstrated that a significant fraction of neuroblastomas (10%) is driven by

MYC, via focal duplication and hijacking of *MYC* enhancers in the 8q24 region without amplification of *MYC* itself, or via translocations of *MYC* downstream of heterologous enhancers [85]. Interestingly, the 8q24 focal duplications encompassed either the AML BENC enhancer or regions in close proximity to the minimal focal duplications described for the *MYC*-LASE lung enhancer and the N-Me T-ALL enhancer [38, 39, 47, 67]. Thus, this is the first study suggesting that tissue-specific enhancers of *MYC* can also be hijacked by cancer cells from a different tissue of origin to drive increased *MYC* expression.

• Lung Cancer

Lung Cancer is one of the most frequent cancers and the leading cause of cancer related death in the world [58]. A recent study identified amplifications of non-coding regions near the *MYC* gene, with a focal amplification peak located 450Kb downstream of *MYC* in lung adenocarcinoma [67]. This peak, named *MYC*-LASE (for *MYC* Lung Adenocarcinoma Super-Enhancer) encompasses a 23Kb non-coding region with high levels of H3K27ac that is part of a broader super-enhancer region. Notably, *MYC*-LASE is able to loop and interact with the *MYC* promoter in A549 lung adenocarcinoma cells. Interestingly, 17% of primary lung adenocarcinoma cases have focal amplifications of *MYC*-LASE co-occurring with *MYC* amplification, whereas 2% of cases have focal amplifications of *MYC*-LASE without concomitant amplification of *MYC* [67], highlighting its relevance in this disease. Moreover, primary cases with *MYC*-LASE amplification showed increased transcriptional levels of *MYC* and CRISPR/Cas9-mediated experiments in cell lines in which *MYC*-LASE was either repressed or deleted resulted in the transcriptional downregulation of *MYC* and decreased growth *in vitro* [67].

In addition, duplications encompassing the whole *MYC* gene, as well as upstream regulatory regions with H3K27ac marks, have been also detected in small cell lung cancer (SCLC) [20, 86], suggesting that these upstream areas might also play an important role in the regulation of *MYC* in other types of lung cancer.

• Uterine and Ovarian Cancer

Uterine cancer is the fourth most common type of cancer in women with 61,000 new cases in 2017, whereas ovarian cancer accounts for more deaths than all other gynecological cancers combined [58]. The SNP rs10088218 (chr8:128,531,703) has been associated to increased ovarian cancer risk [87], however, functional data linking it to increased *MYC* levels is still lacking. Interestingly, the same study that identified *MYC*-LASE in lung adenocarcinoma also detected frequent noncoding amplifications near the *MYC* gene in uterine cancer samples, and named this region *MYC*-ECSE (for *MYC* Endometrial Cancer Super-Enhancer) [67]. *MYC*-ECSE encompasses a 10Kb super-enhancer region located ~800Kb downstream of the *MYC* gene transcription start site, while *MYC*-LASE is located ~450Kb downstream of *MYC*. Similar to *MYC*-LASE, *MYC*-ECSE is able to loop and interact with the *MYC* promoter in Ishikawa endometrial carcinoma cells. In addition, approximately 10% of uterine cancer patients had focal amplifications of both *MYC*-ECSE and *MYC*, whereas 4% of cases had focal amplification of only *MYC*-ECSE and both settings correlated with increased transcriptional levels of *MYC*, highlighting the importance of this region for driving *MYC* expression in uterine cancer [67].

• Glioma

Gliomas accounts for ~80% of all primary malignant brain tumors and most low-grade and secondary high-grade gliomas harbor mutations in IDH1 or IDH2 [88]. Several SNPs at 8q24 have been associated with increased risk of glioma formation [89, 90]. Among these SNPs, the rs55705857 variant (chr8:129,633,446), located downstream of *MYC* in a non-coding intronic region of the *CCDC26* gene, is the most strongly associated with IDH-mutated gliomas [91]. Interestingly, it has recently been shown to confer increased *MYC* activity using reporter assays [89], although further studies are still needed to confirm its potential effect on *MYC* transactivation *in vivo*.

4. Concluding remarks

The functional characterization of cancer-specific enhancers is still in its early stages, however, *MYC* has been shown to be critically regulated by a plethora of tissue-specific regulatory regions (Table 1), underscoring the fundamental importance of enhancers in cancer and making of *MYC* the best example yet of long-range regulation (Figure 1). Moving forward, key questions remain (see also Outstanding Questions) such as how to therapeutically target *MYC* taking advantage of these specific enhancers. Different reports in literature so far suggest that BRD4 inhibition, which disproportionately binds to enhancer regions, results in decreased *MYC* expression [78]. However, ways to more specifically target discrete enhancers are still to be identified. To this end, we might still need to first uncover and categorize all the specific components required for the activity of each particular super-enhancer. Moreover, it is still unclear what might be the relative increase in *MYC* expression in cases with *MYC* amplification alone *vs.* cases with *MYC*-enhancer amplification alone or cases with concomitant amplification of both *MYC* and *MYC*-enhancer regions. Given that *MYC* levels are affected not only by amplifications of its coding region or its enhancers, but also many other different factors and pathways that converge on *MYC*, it is difficult to provide a clear answer. We can only speculate that *MYC*-enhancer amplification might conceivably lead to a lesser increase in *MYC* levels as compared to cases with amplification of the *MYC* coding sequence. Still, cases with *MYC*-LASE or *MYC*-ECSE amplification only compared to cases with *MYC* amplification alone showed comparable levels of *MYC* upregulation [67]. Large cohorts of patients with *MYC* amplifications *vs.* patients with *MYC*-enhancer amplifications would still be needed for each particular tumor type to shed additional light on this issue. In turn, this might have important implications for the phenotypes observed since cancer cells respond to very slight changes in *MYC* expression, and different levels of *MYC* upregulation might even have opposite effects in terms of apoptosis and/or cell growth and proliferation [26–28]. In addition, the basic mechanisms driving enhancer-promoter transactivation are still unclear. In this context, a very recent study suggests that most, if not all, of the enhancers controlling *MYC* expression work via looping to a common docking site located 2Kb upstream of the *MYC* promoter and showing CTCF binding [22]. However, an independent study in which this CTCF docking site was specifically mutated in mice showed very limited effects on *Myc* expression [64], calling into question its relevance *in vivo*. This discrepancy might be explained by inter-species differences in the use of this CTCF binding site or by compensatory mechanisms driving new enhancer-promoter interactions in the absence of this docking site, but further

experiments are still needed to reconcile these findings. In addition, the newly identified mechanism of competition between *MYC* and *PVT1* for enhancer looping in breast cancer adds a novel layer of regulation between enhancers and promoters, and reveals an unexpected insulator function to the *PVT1* gene promoter [65]. Similarly, the functional significance of the cross-talk between different *MYC* enhancers, such as the BENC and N-Me enhancers, remain to be fully elucidated [39]. Another critical question in the field is whether some of these tissue-specific enhancers of *MYC* can be aberrantly activated and hijacked in other cancer types where they are not supposed to be functional and, indeed, this has just been shown to be the case at least for some neuroblastoma cases [85]. However, it is still unclear how this hijacking might happen at the mechanistic level and it is also currently unknown whether this phenomenon might happen more broadly with other enhancer regions as well. Similarly, it would be also interesting to address whether enhancers of *MYC* that have been recently identified to be involved in facial morphogenesis might also be implicated in the development of certain types of cancer [92]. Finally, the mechanisms that dictate the tissue-specificity and the priming and activation of enhancers are yet to be fully characterized. Some reports have suggested that the pioneering activity of lineage-specific transcription factors might be involved in the activation of specific enhancers [93, 94], still, further studies are needed to confirm this hypothesis and to identify the transcription factors ‘opening’ each of these enhancer regions of *MYC*.

Overall, all of these results highlight the importance of long-range transcriptional regulation of *MYC* in cancer, and justify the efforts to further dissect the “enhancer-ome” of *MYC*.

Acknowledgements

We apologize to authors whose work could not be cited due to space constraints. This work was funded by the National Institutes of Health/National Cancer Institute grant K99/R00 CA197869 (to D.H.), by an Alex’s Lemonade Stand Foundation Shark Tank Award (to D.H.), and by a Leukemia Research Foundation New Investigator Award (to D.H.).

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Trends Box

1. Numerous mechanisms can lead to *MYC* upregulation in cancer and typically involve gene duplications or translocations
2. Recent studies have identified a plethora of tissue-specific long-range enhancers of *MYC* that control its transcription in a tightly regulated-manner
3. These tissue-specific *MYC* enhancers are involved in normal development, in tumorigenesis or in both processes.
4. Deregulation of these non-coding *MYC* enhancer regions constitutes a novel mechanism leading to *MYC* hyperactivation in different cancer types and might involve: (i) duplications; (ii) disease-associated SNPs; (iii) *de novo* enhancer acquisition or hijacking; or (iv) mutations in the promoter of other genes competing for the same enhancer regions.

Outstanding Questions

1. How can we selectively target *MYC* taking advantage of these tissue- and cancer-specific enhancers?
2. What is the quantitative difference in *MYC* upregulation obtained by amplification of *MYC* alone, amplification of a *MYC*-enhancer alone or concomitant amplification of both?
3. What are the basic mechanisms controlling enhancer-promoter transactivation?
4. Can tissue- and cancer-specific enhancers be hijacked by independent cancer types?
5. What are the mechanisms that dictate the tissue- and cancer-specificity of these enhancers, as well as their priming and activation?

Figure 1. Graphical representation of the different enhancers of *MYC* located in the *MYC* TAD on chromosome 8q24. Different colors refer to different cancer types where the respective enhancers play a role. Enhancer abbreviation names refer to the names given by the authors who originally described them.

Table 1:

Detailed explanation of enhancer modifications involved in different cancer types, with coordinates and references (MYC coordinates: 127,734,069–127,743,434 (hg38)).

Cancer type	Location in 8q24 (hg38)	Name of the region	Modifications in cancer	Functional validation	References
Prostate Cancer	127,062,671–127,162,671	Enhancer region 2	Duplications	No	[39, 40, 43, 44, 49, 51, 52]
	127,389,527–127,458,527	Enhancer region 3	Duplications	Mouse model <i>in vivo</i>	[40, 43, 44, 48–52]
			SNP rs6983267 (127,401,060)	Mouse model <i>in vivo</i>	[43, 46, 49]
			Duplications	No	[39, 40, 43, 44, 48, 49, 51, 52]
B-Cell Malignancies (CLL, SLL and MCL)	127,117,755–127,287,755	B-NDME	SNP rs1447295 (127,472,793)	No	[40]
			Duplications	Cell lines <i>in vitro</i>	[21–23]
			SNP rs2466024 (127,175,774)	No	[20]
			SNP rs2456449 (127,180,736)	No	[20]
			Not described	Mouse model <i>in vivo</i>	[39, 53]
Breast Cancer	127,337,755–127,497,755		SNP rs13281615 (127,343,372)	No	[41]
			Mutations in the <i>PVT1</i> promoter	Cell lines <i>in vitro</i>	[54]
Colorectal Cancer	127,389,527–127,458,527	Enhancer region 3	Not described	Mouse model <i>in vivo</i>	[39, 49, 53, 62]
			SNP rs6983267 (127,401,060)	Cell lines <i>in vitro</i>	[42, 61]
Medulloblastoma	127,629,810–127,655,034		Duplications	No	[65]
Merkel Cell Carcinoma (MCC)	127,687,755–127,987,754		Not described	No	[67]
Pancreatic Cancer	127,813,000–127,963,000		Duplications	No	[11, 69]
Clear Cell renal Cell Carcinoma (ccRCC)	127,875,000–127,878,526	HRE	SNP rs35252396 (127,877,376)	Cell lines <i>in vitro</i>	[70, 71]
Acute Myeloid Leukemia (AML)	127,917,270–127,918,105	ME1	Not described	Cell lines <i>in vitro</i>	[38]
	128,251,139–128,251,614	ME2	Not described	Cell lines <i>in vitro</i>	[38]
	129,546,728–129,712,179	BENC	Duplications	Mouse model <i>in vivo</i>	[34–38]

Cancer type	Location in 8q24 (hg38)	Name of the region	Modifications in cancer	Functional validation	References
Neuroblastoma	128,102,499–128,117,052	Surroundings of <i>MYC</i> -LASE focal amplifications	Duplications	No	[74]
	129,213,258–129,666,373	Surroundings of N-Me focal amplifications	Duplications		
Lung Cancer	128,154,301–128,178,044	BENC			[56]
		<i>MYC</i> -LASE	Duplications	Cell lines <i>in vitro</i>	
Uterine and Ovarian Cancer	128,531,703–128,542,048	<i>MYC</i> -ECSE	Duplications	No	[56]
			SNP rs10088218 (128,531,703)	No	
Facial Morphogenesis	128,688,000–129,328,000	MNE NEE	Not described	Mouse model <i>in vivo</i>	[82]
			rs987525 (128,933,908)	No	
T-Cell Acute Lymphoblastic Leukemia (T-ALL)	129,149,529–129,189,766	N-Me	Duplications	Mouse model <i>in vivo</i>	[27, 28, 30–32]
Glioma	129,633,091–129,633,776		SNP rs55705857 (129,633,446)	Cell lines <i>in vitro</i>	[78, 80]