Myc Goes Global: New Tricks for an Old Oncogene

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Abstract

Myc, a transcription factor commonly deregulated in tumorigenesis, is thought to mediate its diverse cellular effects by altering the expression of specific target genes. However, it has been difficult to gain a precise understanding of how Myc drives cancer because Myc acts rather weakly at many of its target loci, and it has been reported to regulate as many as 10% to 15% of all cellular genes. A new perspective on this issue has been provided by a recent study that revealed Myc can regulate chromatin structure in a global fashion. These findings suggest actions for Myc that extend beyond the traditional concept of a targeted gene regulator. [Cancer Res 2007;67(11):5061–3]

Myc, the Supermodel of Oncogenic Gene-Specific Transcription Factors

Myc was one of the first oncogenes identified and has subsequently been linked with a wide spectrum of human cancers. However, the molecular mechanisms by which myc promotes tumorigenesis have remained surprisingly elusive (1). In the classic "gene-specific" model, growth factors rapidly induce expression of myc family genes (c-myc, N-myc, and L-myc) encoding basic-helix-loop-helix zipper (bHLH) transcription factors that dimerize with Max. The Myc-Max dimers bind to the E-box sequence CACGTG in the promoters of specific target genes and stimulate their transcription. The protein products of these target genes go on to mediate the downstream effects of Myc on cell biology. Myc is then rapidly degraded, and the pathway switches to a transcriptionally repressive state when Max dimerizes with a group of related bHLH proteins, the Mad family, that are thought to operate as Myc antagonists. The Mad-Max dimers bind the same E-boxes that were previously occupied by Myc-Max dimers and repress transcription.

The molecular mechanism of transcriptional activation by Myc involves recruitment of a co-activator complex containing TRRAP and histone acetyl transferases (HAT) such as GCN5 (2, 3) and TIP60 (4). The HATs acetylate NH2-terminal lysines (K) of chromosomal histones specifically in the vicinity of the transcriptional start site. According to a substantial literature distilled into the histone code hypothesis (5), acetylation of K residues in histones is associated with a more open chromatin structure, shutting down expression of target genes. Thus, these studies support the notion of a chromatin-based molecular switch for gene transcription with the "on" or "off" state determined by the relative concentration of Myc and Mad proteins. In this model, during tumorigenesis, excess Myc leads to a preponderance of Myc-Max dimers and aberrantly elevated expression of specific target genes.

In the context of the "gene-specific" model, it was thought that identifying a handful of specific Myc target genes should unravel the normal function of Myc and its role in tumorigenesis. Thus, taking advantage of the advent of expression microarray technology, many groups set out to identify Myc target genes, and there was great excitement when potential target genes were initially characterized. But unexpectedly, these studies and the many that followed produced a seemingly never-ending stream of putative Myc target genes that eventually numbered in the thousands, often with little overlap in targets identified by different groups (reviewed in ref. 8). It was difficult to reconcile these findings with the dominant "gene-specific" model of Myc as a classic transcription factor. Faced with thousands of potential Myc targets, how could one possibly pick the proverbial needle from the haystack and find the key few genes that mediated the tumorigenic function of Myc? Did they even exist? Were all these genes actually regulated by Myc or were the microarray experiments somehow picking up genes that were mostly not bona fide Myc targets?

Although the expression microarray studies aimed at defining Myc target genes identified a remarkable and somewhat bewildering number of mRNA transcripts whose concentrations were altered in cells with manipulated Myc levels, efforts were also under way to approach the same question in a different, more direct manner by identifying where Myc was bound to chromatin in the genome. Distinct functional genomics approaches in mammalian and fly cells yielded a common, compelling conclusion that generally fit with the microarray studies: Myc bound very ubiquitously throughout the genome, apparently to tens of thousands of genomic sites encompassing up to 15% of all genes but also including some intergenic sites (9–13). The expression of many of the bound genes also seemed to be influenced by Myc, suggesting the binding was functionally meaningful. Surprisingly, the most recent genomics study found that approximately half of all Myc-bound sites were intergenic (>10 kb away from transcriptional start sites), hinting at a potential non-transcriptional function for Myc on chromatin (11). Nonetheless, together, the expression microarray and genomics approaches convincingly challenged the "gene-specific" model that Myc acted strictly by regulating a small number of specific target genes. However, no cogent new model emerged that adequately explained the functional significance of Myc apparently acting so globally.

Myc Regulates a Global Chromatin Program

Before the genomics studies, our mouse knockout studies of N-Myc in neural stem cells were yielding some unexpected results.
Along with the findings that N-Myc was essential for neural progenitor proliferation and brain growth (14), N-Myc null cells exhibited surprising, striking alterations in nuclear structure including decreased size, altered shape, and unusual staining suggestive of broad chromatin condensation (15). These changes seemed to be independent of potential intermediate effects of loss of Myc on cell biology such as proliferation, differentiation, apoptosis, and senescence. Why did the loss of N-Myc dramatically alter nuclear structure? We theorized that Myc was normally required to maintain large domains of active euchromatin, and that targeted disruption of Myc was leading to a widespread heterochromatin-like state. In testing this hypothesis, we found that Myc null cells exhibited a pattern of global histone modification changes consistent with widespread inactivation of chromatin structure leading to a chromatin state sharing some features with heterochromatin. Myc-deficient chromatin was enriched for histone H3 that was dimethylated and trimethylated at Lys 9 (MeK9-H3), marks linked to a repressed chromatin state (16), and Myc null cells exhibited a disrupted nuclear localization of HP1α, which binds MeK9-H3. Myc nulls also exhibited substantial decreases in marks enriched in active chromatin: triMeK4-H3, acetylated K9-H3, and histone H4 acetylated at all NH2-terminal K residues. The acetylation changes with loss of Myc were phenocopied by overexpression of Mad, in support of the model of Myc and Mad antagonism but extending it to global chromatin. Together, the changes in histone modifications supported by electron microscopy and micrococcal nuclease sensitivity assays in N-Myc–deficient cells supported the notion that these cells had undergone a striking expansion of more condensed chromatin structure due to loss of Myc.

How was Myc impinging on global chromatin? The genomics studies on Myc, defining strikingly widespread binding by Myc, suggested a model in which Myc influenced global chromatin structure directly through its DNA binding dependent recruitment of chromatin-modifying activities to tens of thousands of genomic sites. However, as appealing as that direct model was, it remained possible that an indirect mode of action involving specific Myc target genes was at work. In support of this, we also found evidence for a role for a specific target gene, GCN5 (15). The direct and indirect models are presented in Fig. 1. It is important to note that

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Figure 1. Classical gene-specific and new global chromatin models of Myc-induced tumorigenesis. In the classic gene-specific model, Myc binding to DNA is finite and limited to genes whose expression Myc regulates. Once their expression is modulated by Myc, these genes then mediate the downstream effects of Myc on cell biology. In the new global model, Myc binding is widespread encompassing hundreds or thousands of sites per chromosome and including both genic and intergenic sites. This global binding regulates widespread chromatin structure and in that way directs cell biology through influencing functions such as cell cycling and fate that depend on overall chromatin structure. Two potential dual models are also possible. First, Myc may operate in a gene-specific manner but at thousands of genic sites. In this way of thinking, Myc would be acting both globally and in a gene-specific manner without significant intergenic binding. Second, if GCN5 operates not only as a cofactor for Myc but also as a target gene, it may bridge the models supporting a dual model. Each line on a hypothetical chromosome represents a Myc binding event.
two potential dual models may also be operating both direct and indirect mechanisms. In the first dual model, Myc globally binds DNA but only at genic sites. In the second dual model, Myc influences chromatin both directly and indirectly via regulating the expression of the HAT GCN5, which in turn globally acetylates chromosomal histones. The notion of GCN5 as a Myc target gene as well as a Myc cofactor also suggests a feed forward loop whereby Myc regulates expression of its own cofactor.

In addition to recruiting HATs, the Eisenman lab has very recently characterized a family of Myc-associated factors that modulate histone methylation (17). Myc binds and inhibits trithorax proteins (Lid, Rbp2, and Plu-1) that act as demethylases targeting MeK4-H3. In this way, Myc blocks demethylation of MeK4-H3 maintaining an active chromatin mark. These findings fit nicely with our observations of loss of Myc, leading to a profound loss of MeK4-H3, and suggest a novel mechanism by which Myc may influence global chromatin structure. Myc has also recently been found to influence DNA methylation through recruitment of Dnmt3a to block activation by Miz-1 consistent with the ability of Myc to also act as a repressor, an activity linked to transformation (18, 19). However, there is currently no evidence that Myc can globally influence DNA methylation.

Future Perspectives: Myc, Global Chromatin Structure, and Cancer

Although our Myc loss of function studies suggested that Myc was essential for a normal balance of euchromatin and heterochromatin by widely maintaining euchromatin, we also conducted gain of function studies that implied a potential role for Myc and global histone modifications in cancer. Overexpression of Myc in cultured neural stem cells consistently led to histone hyperacetylation. In addition, enforced exogenous N-Myc in the embryonic ventricular zone of nestin-N-Myc transgenic mice was associated both with hyperplasia and histone hyperacetylation. The link between Myc driven preneoplastic changes and global histone acetylation changes suggests that in tumors with deregulated Myc, global histone acetylation changes may become important, a possibility we are now addressing using genomics. Given the relatively few molecules of Myc reported to be present in some cell lines (20), our findings would suggest that each Myc-Max dimer may be endowed with the ability to influence surprisingly large chromatin domains. The high level of Myc molecules present in tumors may effectively lock in an aberrantly active overall chromatin state that is highly resistant to modulation.

Several recent studies by other groups also support the general notion that global histone modification changes may play a role in tumorigenesis (21–23). One puzzling aspect of the findings on Myc, leading to global histone hyperacetylation linked with hyperplasia, is that they seem to contradict the growing evidence of the efficacy of HDAC inhibitor therapies in some cancers (reviewed in ref. 24). The fact that both an excess of the Myc oncogene and treatment of cancer cells with HDAC inhibitors cause global histone hyperacetylation (25) implies that the anticaner function of HDAC inhibitors may not be strictly epigenetic and may very well function via inducing differentiation or apoptosis. It also suggests a highly complex relationship between global histone modification states and cancer. Nonetheless, it is intriguing to speculate that there may be specific overall chromatin structural and histone modification states that play roles in cancer. Such “histone codes of cancer” could form the basis for future new epigenetic therapies based on targeting the chromatin-modifying enzymes that mediate the modifications associated with tumors. Altered global chromatin structure represents a novel mechanism of oncogenic transformation that other oncoproteins are likely to employ. Efforts on many fronts, particularly the cancer epigenome project (25), should provide the answers to the open questions outlined in this review and pave the way for future epigenetically based cancer diagnostics and therapies.

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References

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