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Pathobiology

Transcriptional Regulatory Network of MYC, HIF-1 and Their Target Genes

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Prediction of gene regulatory sequences using interspecies comparative sequence analysis has advanced considerably; however, experimental validation is still lacking. Here, we sought to determine whether putative transcription factor binding sites predicted by manual sequence alignments through dot-plot analysis or the Trafac server could be validated by chromatin immunoprecipitation. In particular, we chose MYC as a model transcription factor to determine whether it binds target glycolytic genes that are predicted by interspecies comparison for canonical MYC binding sites or E-boxes, 5'-CACGTG-3'. Conserved canonical E-boxes in ENO1, LDHA, and HK2 occur in 31-111 bp islands with high interspecies sequence identity (>65%). These conserved regions were all well-bound by MYC in the human P493-6 B lymphocytes. We also sought to determine whether MYC could bind non-conserved canonical E-boxes found in the remaining human glycolytic genes. MYC bound PFKM, but its binding to GPI, PGK1 and PKM2 is diminished. Binding to BPGM, PGAM2 and PKLR were absent. Both GAPD and TPI1 do not have conserved E-boxes, but are induced and bound by MYC through regions with non-canonical E-boxes. This critical evaluation indicates that conserved canonical MYC E-boxes are well-bound in ChIP assays and non-conserved E-boxes have diminished or absent binding. We found that unpredicted regions with non-canonical E-boxes could also bind MYC well, demonstrating a limitation of interspecies sequence comparisons. Our study also suggests that MYC is a global regulator of glycolytic genes, suggesting that MYC plays a key role in a switch to glycolytic metabolism during cell proliferation.

When a tumor reaches a certain size, the microenvironment of tumor mass becomes hypoxic. To overcome and adapt to hypoxic conditions, cancer cells are capable of enhancing glycolysis. It is well established that hypoxia inducible factor-1 (HIF-1) enhances glycolysis in response to hypoxia in both physiologic and pathologic conditions. Glycolysis is also enhanced through the activation of specific oncogenes, such as an oncogenic transcription factor, MYC. The MYC plays a central role in tumor development through binding to canonical MYC binding sites, E-boxes (5'-CACGTG-3') and transactivating a set of target genes. It is quite intriguing that HIF-1 DNA binding sites (5'-RCGTG-3') are similar to canonical MYC binding sites (5'-CACGTG-3') or E boxes. Thus, it would be reasonable to expect that the transcriptional regulatory network of MYC and HIF-1 plays a key role in adapting to hypoxic microenvironment. However, possible roles of a MYC / HIF-1 network in regulating glycolytic gene expression remain to be determined. Moreover, in vivo MYC / HIF-1 DNA binding activity to the common-cis element (5'-RCGTG-3') is poorly understood.

Interestingly, expression (and enzymatic activity) of hexokinase 2 (HK2) was significantly enhanced in P493 cells expressing both MYC and HIF-1 as compared to cells expressing either MYC or HIF-1 alone. Cellular glycolytic rate was also significantly enhanced by MYC and HIF-1. These data suggest that MYC and HIF-1 are coordinating to promote the transcription of HK2 in response to hypoxic condition. To address the molecular mechanism of enhanced expression of HK2, we characterized the DNA binding activity of MYC and HIF-1 by phylogenetic footprinting analysis and a scanning ChIP assay. In HK2 gene, MYC bound highly conserved E boxes in intron 1, whereas the HIF-1 binding site(s) appear to be in the promoter region, which is at least 1kb away from bona fide intronic MYC binding sites.

In summary, our study demonstrates that cancer cells are capable of enhancing the expression HK2 through modulating MYC / HIF-1 transcriptional regulatory network. Increase in HK2 promote aerobic tumor glycolysis, termed the Warburg effect, which is a critical step to adapt to hypoxic microenvironments. Further delineating DNA binding activity of these transcription factors will extend our understanding of connection between MYC / HIF-1 network and cellular metabolic homeostasis in hostile microenvironment. In particular, our ongoing study will provide evidence of how two transcription factors sharing a common binding site modulate their DNA binding activities.