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Mutational and functional analysis of MGA, a MYC antagonist gene, in lung cancer

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Introduction: MGA gene encodes a heterodimeric partner of the MYC-interacting protein MAX. MGA interacts with MAX through their basic region/helix loop helix/leucine zipper (bHLHZ) domains to bind to the E-boxes of the DNA which results in repression of target genes. In addition to bHLHZ domain, MGA contains a T-box domain that recognizes the Brachyury binding sequence, which hints the possibility of a MAX-independent mechanism for DNA-binding (1). Recent meta-analysis revealed that MGA is mutated in lung adenocarcinomas. Most of these are loss-of-function mutations which raise the possibility that MGA functions as tumor suppressor (2, 3). Since MGA mutations are mutually exclusive with alterations at other members of the MYC-pathway, including MYC itself, it has been proposed a role of MGA in the control of MYC oncogenic activity.

Objectives: Here, we aimed to characterize the role of MGA in lung adenocarcinoma development. Specifically we focused in studying MGA's role in modulating the MYC pathway by identifying genes functionally related to MGA. We also intended to analyze the influence of MGA in the regulation of MYC genetic pathway.

Methodology: We used chromatin immunoprecipitation-sequencing (ChIP-seq) and RNA-sequencing (RNA-seq) analysis to identify MYC and MGA DNA binding sites and binding motifs. We combined these results with immunoprecipitation assays and mass spectrometry (IP-MS) to elucidate the mechanism of the gene repression activities mediated by MGA. We also determined the capability of MGA overexpression to affect cell proliferation, using a cell competition assay. Finally, we used an electrophoretic mobility shift assay (EMSA) to evaluate the effect that missense mutations in the bHLH domain of MGA have in its binding ability to the DNA.

Results: IP-MS analysis reported the interaction of MGA with the non-canonical polycomb repressive complex 1 (ncPRC1), histonede-acetylases HDAC1/2 and the E2F6 transcriptional repressor, suggesting a potential mechanism by which MGA represses its target genes. These interactions were further validated by co-IP assays. Furthermore, we found that ectopic expression of wild type MGA represses the cellular growth of lung adenocarcinoma cell lines. ChIP-seq and RNA-seq results evidenced that MGA recognizes the same DNA binding motif as MYC, that it shares a large proportion of genomic DNA binding sites with MYC, and that it represses the expression of MYC-target genes. Through the search in the Pan-cancer database, we found that the bHLH domain of MGA presents recurrent missense mutations in colorectal and in endometrial cancer. EMSA assays showed that these mutations impair the DNA binding ability of MGA to the DNA, disrupting the function of MGA in cancer cells.

Conclusions: (I) MGA has been found mutated in multimple types of cancer, including lung cancer. (II) Loss-of-function mutations disrupt the function of MGA in cancer cells. (III) As part of the noncanonical PRC1 repressive complex, MGA participates in the control of gene expression that is abrogated in MGA-mutant cancer cells. (IV) MGA acts as a repressor for the MYC pathway by binding to and repressing genes that are bound and activated by the MYC oncoprotein. Altogether, the abrogation of these gene expression repressive activities might underlie the tumor suppressor role of MGA.

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