The c-MYC oncoprotein, the NAMPT enzyme, the SIRT1-inhibitor DBC1, and the SIRT1 deacetylase form a positive feedback loop

Antje Menssen1, Per Hydbring2,3, Karsten Kapelle2, Jörg Vervoorts4, Joachim Diebold4, Bernhard Lüscher2, Lars-Gunnar Larsson5, and Heiko Heremeking6,1

1Experimental and Molecular Pathology, Institute of Pathology, Ludwig-Maximilians-University Munich, D-80337 Munich, Germany; 2Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, 171 77 Stockholm, Sweden; 3Institute of Biochemistry and Molecular Biology, Medical School, Rheinisch-Westfälische Technische Hochschule, Aachen University, D-52074 Aachen, Germany; and 4Institute of Pathology, Kantons Spital Luzern, 6000 Luzern 16, Switzerland

AUTHOR SUMMARY

The c-MYC oncogene is aberrantly activated in most human tumors, endowing cancer cells with the capacity for infinite division, also known as immortalization. The SIRT1 enzyme, which inactivates numerous inhibitory proteins and thereby extends cellular life span, seemed to have potential as a mediator of c-MYC functions. We found that c-MYC activates SIRT1 by enhancing the production of its cofactor NAD+ as well as by sequestering DBC1, an inhibitor of SIRT1. Furthermore, SIRT1 itself activates c-MYC, creating a positive feedback loop that may spin out of control in cancer cells that continuously produce c-MYC. Fig. P1 summarizes our findings.

It is still largely unknown how c-MYC converts normal cells into tumor cells that have limitless proliferation capacity and enhanced robustness, even under adverse growth conditions. The c-MYC proto-oncogene (i.e., a normal gene with the capacity to become a cancer-causing oncogene) encodes the c-MYC transcription factor, which regulates a large array of target genes. In general, transcription factors are proteins that bind to specific sections of the genome (the complete genetic set) and activate or inhibit certain genes. We analyzed the effect that experimental c-MYC activation had on the expression of SIRT1 protein and mRNA. As the activity of SIRT1 depends on the cofactor NAD+, we also investigated the effects of c-MYC on metabolic pathways that generate NAD+. Because we have previously observed that the SIRT1 inhibitor DBC1 binds to c-MYC (1), we tested whether the binding of c-MYC to DBC1 may positively influence SIRT1 activity. As SIRT1 regulates the activity of many nuclear proteins, we asked whether SIRT1 may also regulate c-MYC and, if so, how.

To study the putative role of SIRT1 after c-MYC activation, we first analyzed the mRNA transcript and protein levels of SIRT1 upon activation of various versions of the c-MYC gene in several cell types. We also studied the effects of reduced or abrogated expression of c-MYC with a variety of methods (e.g., nutrient starvation, genetic deletion, and the use of shRNA molecules that can bind specific genetic segments in the c-MYC mRNA that block its function). After c-MYC activation, the expression of SIRT1 mRNA and protein was compared with genes known to be regulated by c-MYC (2). The correlation between c-MYC and SIRT1 expression was also studied in colorectal cancer specimens. Following c-MYC activation, we detected increased SIRT1 protein levels and activity without any concomitant mRNA increases. This indicated that c-MYC induces the activation of SIRT1 on the protein level and not by simply inducing SIRT1 gene expression. Furthermore, inactivation of c-MYC decreased the amount of SIRT1 protein.

We also studied the NAD+ salvage pathway, a metabolic pathway necessary to provide NAD+ for SIRT1 activation. It is regulated by the rate-limiting...
enzyme NAMPT (3). Therefore, we tested a putative involvement of NAMPT in SIRT1 activation downstream of c-MYC by chemically inhibiting NAMPT, then measuring levels of NAD$^+$ and its reduced form, NADH. In addition, we analyzed the relevance of this SIRT1 activation by experimental inhibition of SIRT1 expression and activity. We also tested whether an increase of c-MYC protein reduces DBC1 binding to SIRT1 and thereby relieves the repression of SIRT1. Furthermore, the c-MYC/SIRT1 protein interaction and its functional consequences were analyzed. Finally, we studied the SIRT1-mediated deacetylation of the c-MYC protein and how this affects the stability and activity of c-MYC.

Our analyses establish a positive feedback connecting c-MYC, NAMPT, DBC1, and SIRT1. First, we identified NAMPT as a gene directly targeted by c-MYC, and an important mediator of SIRT1 activation upon c-MYC induction. Although an NAD$^+$ increase following NAMPT induction can explain SIRT1 activation, the molecular mechanism that leads to increased SIRT1 protein levels remains unknown. Second, we found evidence that the binding of c-MYC to the SIRT1 inhibitor DBC1, which leads to sequestration of DBC1 from SIRT1, may contribute to SIRT1 activation in cells with elevated c-MYC expression. Upon simultaneous inactivation of SIRT1 and activation of c-MYC, cells underwent a higher rate of cell death, also known as apoptosis. Therefore, SIRT1 is presumably required to suppress c-MYC–induced apoptosis. Furthermore, we found that SIRT1 directly binds to c-MYC protein and modifies it by deacetylation (that is, by removing an acetyl moiety, a common mode of regulating protein functions). The SIRT1-modified c-MYC thus produced has increased levels of lysine 63-linked ubiquitin chains, features that may stabilize it. Overall, the SIRT1-mediated deacetylation of the c-MYC protein stabilizes c-MYC and enhances activation of c-MYC’s target genes.

The positive feedback regulation among c-MYC, NAMPT, DBC1, and SIRT1 described here provides an important insight into the function and regulation of the prototypic c-MYC oncogene. Our results present a direct link between the NAD$^+$ salvage pathway and c-MYC. The c-MYC–induced increase in NAD$^+$ levels may affect additional cellular functions. Finally, our study suggests that tumor cells with deregulated expression of c-MYC may be especially sensitive to inhibition of the NAMPT and/or SIRT1 enzymes, opening the door to additional therapeutic treatments for cancer.