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Review

Myc induced replicative stress response: How to cope with it and exploit it☆

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ABSTRACT

Myc is a cellular oncogene frequently deregulated in cancer that has the ability to stimulate cellular growth by promoting a number of proliferative and pro-survival pathways. Here we will focus on how Myc controls a number of diverse cellular processes that converge to ensure processivity and robustness of DNA synthesis, thus preventing the inherent replicative stress responses usually evoked by oncogenic lesions. While these processes provide cancer cells with a long-term proliferative advantage, they also represent cancer liabilities that can be exploited to devise innovative therapeutic approaches to target Myc overexpressing tumors. This article is part of a Special Issue entitled: Myc proteins in cell biology and pathology.

1. Introduction

c-Myc is a transcription factor acting as a master regulator of genes involved in cell cycle progression, cell growth, differentiation, metabolism and apoptosis. It is also a potent cellular oncogene that is found frequently deregulated in human cancers. This pathological upregulation is frequently due to chromosomal translocations leading to promoter rearrangement [1–5], gene amplification [6,7] or viral mediated insertional mutagenesis [8,9]. Alternative mechanisms of deregulation found in cancers are represented by point mutations in the coding region, potentially leading to gain of function [10,11] and genetic polymorphisms identified in distant regulatory region(s) which are activated in a tissue and cancer specific fashion [12]. c-Myc belongs to the basic Helix-Loop-Helix Leucine Zipper (bHLHZip) transcription factor family. These domains are located at the C-terminus where the basic region, needed for DNA interaction, is followed by the Helix-Loop-Helix [13] and the Leucine Zipper domains [14]. The latter domains are both required for the interaction with Max, the obligatory Myc partner needed for the formation of the transcriptionally active heterodimers [15,16]. The N-terminal domain possesses transactivating properties [17] and contains four regions called Myc homology boxes which are highly conserved within the other members of the Myc family (i.e. N-Myc and L-Myc). Myc can also repress gene transcription by tethering other transcription factors like Miz-1 at the promoter of selected genes [18–20]. Recent genome wide studies have highlighted the pervasive nature of c-Myc dependent transcriptional programs with an estimated 25,000 genomic sites bound by Myc which include RNA pol III and pol I dependent genes as well as non coding RNAs [21–24]. This global transcriptional regulatory role of Myc accounts for its pleiotropic activity in regulating cellular processes.

In this review we will concentrate on c-Myc ability to regulate DNA replication by providing an overview of how DNA synthesis is controlled during S-phase, describing how the overexpression of c-Myc can ensure a productive DNA synthesis avoiding the pitfalls of replication stress responses and we will conclude by highlighting how this knowledge could be exploited for therapeutic intervention in cancer.

2. Chapter 1. DNA replication in mammals

Faithful cell cycle progression requires that DNA is precisely and efficiently duplicated during S-phase. Given the large size of the metazoan genome, higher eukaryotes had to evolve mechanisms to allow DNA synthesis from a sufficient number of independent replication origins to ensure timely and co-ordinated DNA duplication. Yet, the activity of DNA replication origins has to be controlled in order to ensure that each replication origin would fire only once every cell cycle thus avoiding the re-replication of the genome, which would lead to regional genome amplification. The correct regulation of DNA synthesis is achieved by a process called replication origin licensing, which is established in late mitosis and early G1, well before cells enter S-phase.

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Origin licensing begins with the recruitment of the Origin Recognition Complex (ORC) onto DNA replication origins along with the two licensing factors Cdc6 and Cdt1. Once this complex is loaded it allows the recruitment of the minichromosome maintenance (MCM) complex which is composed of six essential replication proteins (MCM2-7) that are though to act as helicases needed to unwind the template DNA ahead of the replication fork. Overall, this multi-protein complex, termed the pre-replicative complex (pre-RC), is loaded onto DNA before S-phase entry [25,26]. Replication is then initiated by the action of S-phase cyclin dependent kinases and Cdc7, which promotes the binding of Cdc45 and GINS to the pre-RC [27,28]. DNA is then unwound by the Cdc45-MCM2-7-GINS (CMG) complex [29–32], which also allows the loading of DNA polymerase-α to initiate DNA synthesis [33–35]. In order to avoid re-replication the licensing system has to be shut down before entry and during S-phase progression, therefore a tight regulatory mechanism is set in place to prevent intra S-phase re-licensing. This is based on the efficient proteasomal degradation of Cdt1 which is regulated by the S-phase specific activity of the cyclin A/Cdk2 complex and the recruitment of the processivity factor PCNA on the replicative fork. In addition, the expression of Geminin and its nuclear targeting provide an efficient mechanism to inactivate Cdc6 during S-phase. Consistent with this model, the deregulation of either Cdt1 or Cdc6 has been shown to cause DNA re-replication [36]. Given the crucial role of replication forks during DNA replication, eukaryotes have devised a number of safeguarding mechanisms to improve the robustness of the system: not only replication origins are fired in excess over the minimum number required to complete S-phase in a timely manner, but also cells, during G1, load onto DNA an excess of MCM molecules relative to the number of replication origins that will be used during DNA synthesis [37–39]. This renders cells relatively resistant to the fluctuation of the MCM levels [38–40] and provides a reservoir of dormant origins that may be used by cells as a back-up in the event of irreversible stalling of the active forks. Experimental evidences suggest that excess of MCM2–7 is required for cells to properly cope with replicative stress [41–43].

3. Chapter 2. Myc and DNA replication

Since its early identification as an avian oncogene, several observations linked c-Myc to cellular proliferation. Its mRNA was shown to be quickly expressed upon mitogenic stimulation of quiescent cells [44] and kept to steady levels during cell cycle progression [45]. Conversely, anti-proliferative signals were shown to quickly downregulate c-Myc mRNA abundance [46,47]. Moreover, c-Myc expression was not only associated with cellular growth but also with allowed cellular proliferation in reduced serum conditions, in tissue culture cells [48]. In addition, the conditional activation of c-Myc in quiescent cells induced cell cycle entry and promoted cellular proliferation in vitro [49,50] and in vivo [51,52]. c-Myc ability to directly control DNA synthesis is integral to its role in supporting cellular growth. Indeed, overexpression studies have evidenced how ectopic expression or conditional activation of c-Myc triggers an increase in the percentage of S-phase cells in asynchronous populations. This increase is frequently matched by an overall acceleration of the S-phase which is achieved by increasing the number of firing replication origins [53–55].

This ability of c-Myc to promote cellular proliferation stems from its proficiency, as a transcription factor, to directly control the expression of a large number of genes implicated in S-phase entry and progression [56]. First, elevated c-Myc accelerates progression through G1 by positively regulating the expression of several cyclins and cyclin dependent kinases (Cdks) like cyclins D1 and D2, Cdk4, and cyclin B1 [57–60] and many others, as suggested by the recent genome wide studies [21]. This regulation is not only relevant in physiological conditions but also linked to the oncogenic function of c-Myc: in vivo genetic studies have suggested a role for Cdk4 in c-Myc mediated transformation [61], while Cdk2 is required to repress Myc induced senescence [62]. In addition, fibroblasts lacking cyclins D1–3 fail to be transformed by c-Myc, suggesting an important role for D-type cyclins in c-Myc-mediated transformation [63]. On the other hand, c-Myc represses anti-proliferative genes like the Cdk inhibitors p21 [64–66] and $p15^{INK4A}$ [67,68], through an interaction with the Miz-1 protein at the core promoter, and several other genes involved in growth arrest, such as Gadd45 [69] and Gas1 [70]. Thus c-Myc regulates a variety of genes directly involved in cell cycle regulation.

Complementary to this, c-Myc manages the supply chain of metabolites needed for DNA replication by transcriptionally regulating the majority of the genes involved in purine and pyrimidine biosynthesis pathways, these genes not only are bound and transcriptionally regulated in cell lines but also are responsive to c-Myc activation in the liver of transgenic mice [71,72]. Of note, chemical inhibition of the c-Myc targets inosine monophosphate dehydrogenases (IMPDH1 and IMPDH2) results in S-phase arrest and apoptosis suggesting that balancing the nucleotide pool is essential for c-Myc's orchestration of DNA replication, to the point that the uncoupling of these two processes creates DNA replication stress and apoptosis [72]. Rather unexpectedly, c-Myc can also promote S-phase via a transcription independent function. Indeed the c-Myc protein was shown to localize at nuclear sites coinciding with active ongoing DNA replication, possibly representing early replicating origins. This was supported by the identification of high molecular weight complexes containing c-Myc and the pre-RC complex but devoid of proteins involved in DNA replication elongation (MCM10, RPA and PCNA). The use of *Xenopus* extracts which are transcriptionally incompetent, suggested a transcriptional independent function of c-Myc in controlling S-phase progression possibly by directly participating in licensing or assembly of (pre)-replicative complexes [73]. Thus, the S-phase promoting activity of c-Myc relies on its direct activation of the basic cell cycle machinery (cyclins and Cdks), the transcriptional regulation of pathways directly linked to DNA metabolism and direct regulation of the firing of early replisomes. The combined action of these different processes results in a harmonic acceleration of DNA synthesis where the increased number of highly processive replisomes is fuelled by a robust supply of nucleotides.

4. Chapter 3. Origin of DNA damage in Myc overexpressing cells

The overexpression of c-Myc in a number of in vitro cellular systems has been associated with the activation of a DNA damage response (DDR) [74–76] and increased genomic instability [77–81] thus suggesting that elevated c-Myc levels lead to the accumulation of DNA damage. These observations have been complemented by in vivo studies with c-Myc transgenic mouse models showing that tissue specific deregulation of c-Myc is associated with a DNA damage response [74–76]. Although the molecular details are ill defined, it is likely that c-Myc deregulation affects DNA stability in different ways thus possibly leading to the rampant genomic instability observed in some c-Myc dependent tumor models [77–80,82]. Several evidences suggest that c-Myc induced genomic instability is multifactorial.

First Myc over-expression can cause DNA oxidative damage by enhancing mitochondrial biogenesis and cellular metabolism since it may boost cellular metabolism in the absence of the appropriate compensatory mechanisms that normally scavenge free oxygen radicals, thereby exposing cells to elevated ROS which will oxidatively damage DNA [83–85]. This is supported by the observation that the accumulation of reactive oxygen species (ROS) is frequently associated with c-Myc activation [83,86,87].

Another source of genome instability may be related to c-Myc ability to regulate telomere biology. Although, a mechanistic insight is still missing, there is evidence that conditional activation of c-Myc affects the spatial distribution of telomeres in the interphase nuclei leading to a strong aggregation of telomeres. This in turn associates with an increase in the frequencies of end-to-end chromosomes fusions suggesting that this nuclear reorganization affects telomere function by promoting breakage-fusion-bridge cycles [88].

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