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MDM2 as MYCN transcriptional target: Implications for neuroblastoma pathogenesis

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Abstract

MYCN amplification is associated with an exceptionally poor prognosis in neuroblastoma. Furthermore, the crucial effectors of MYCN responsible for this aggressive subset of neuroblastoma await characterization. A critical negative regulator of the p53 tumor suppressor, *MDM2*, has been recently characterized in neuroblastoma cell lines as a transcriptional target of MYCN. Targeted inhibition of MYCN results in reduced *MDM2* expression levels, with concomitant stabilization of p53 and stimulation of apoptosis in MYCN amplified neuroblastoma cell lines. These data suggest the possibility that MYCN-driven expression of *MDM2* might play a role in counterbalancing the p53-dependent apoptotic pathways concurrently stimulated by over expression of MYC proteins. Mouse models of lymphoma have demonstrated that *MDM2* expression, with decreased p53 activity, is critical for complete MYCC driven tumorigenesis. Our data suggest that a similar situation may apply for MYCN in neuroblastoma. Strategies for pharmacologic and genetic inhibition of *MDM2* may prove to be an important new therapeutic approach in neuroblastoma.

Keywords: MYCN; *MDM2*; Apoptosis; Neuroblastoma; p53

1. Introduction and background

Major improvements in outcome for neuroblastoma are most likely to result from targeted molecular therapies, derived from an advanced understanding of the molecular pathogenesis of the disease. MYCN amplification is the strongest adverse prognostic factor in neuroblastoma treatment [1] and transgenic models of neuroblastoma have revealed that MYCN is also a transforming oncogene responsible for de novo tumor formation [2]. MYCN is involved in many aspects of normal and oncogenic cellular physiology *via* activation of various transcriptional targets. These target genes encode proteins with roles in proliferation, cell cycle regulation, apoptosis and genomic stability [3,4]. In vitro studies demonstrate that MYCN overexpression induces an aggressive metastatic phenotype with decreased contact inhibition,

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decreased growth factor dependence and increased proliferation rate [5–7].

Paradoxically, MYCN also suppresses Bcl-2, activates Bax, and sensitizes cells to genotoxicity-mediated apoptosis through intrinsic apoptosis pathways [8]. MYCC (a MYC family member) has also been shown to activate the ARF tumor suppressor leading to p53 activation and apoptosis through Bcl-x and Bcl-2-dependent and independent pathways [9]. Thus MYCN expression induces the apparently contradictory cellular processes of rapid proliferation and apoptotic cell death. Obligate defects in the apoptotic pathways accompanying MYCN amplification have been proposed to circumvent MYCN-stimulated cell death in neuroblastoma [10]. Characterization of the key downstream transcriptional targets of MYCN involved in these conflicting pathways will yield important insight into the pathogenesis of and novel therapeutic approaches to neuroblastoma.

2. *MDM2* is a MYCN target

Using chromatin immunoprecipitation (ChIP) cloning [11], we recently characterized *MDM2*, the essential negative regulator of the p53 tumor suppressor, as a transcriptional target of the MYCN oncogene in neuroblastoma [12]. Real time PCR, Western blots and luciferase reporter assays demonstrate MYCN dependent regulation of *MDM2* in MYCN-inducible neuroblastoma cell lines. ChIP and promoter pull-down assays demonstrate that this transcriptional regulation was through a direct interaction with a canonical E-box in the *MDM2* promoter. This study also shows that targeted inhibition of MYCN leads to decreased *MDM2* and consequently increased p53 mediated apoptosis.

In contrast to many tumors, less than 2% of neuroblastomas have mutated p53 and the p53 pathways are functionally active in the majority of de novo tumors [13,14]. The characterization of *MDM2* as a direct transcriptional target of MYCN suggests a mechanism by which MYCN overexpression might destabilize p53, thereby raising the threshold for stimulation of growth arrest and apoptosis. As detailed below, inhibition of the *MDM2*/p53 pathway in vivo may tip the balance between MYCN driven proliferation and apoptosis in favor of apoptosis through activation of p53.

3. *MDM2* expression as a critical determinant in cancer

Originally identified as an amplified gene located on a double minute chromosome in a transformed mouse cell line [15], Mdm2 was later shown to have a critical role in the process of cellular transformation [16]. Amplification and overexpression of *MDM2* is found in about 10% of all human tumors [17]. In gliomas, for example, *MDM2* amplification identifies a subset of high-risk patients that do not have p53 mutations [18]. In many soft tissue sarcomas, *MDM2* amplification and overexpression correlates with poor prognosis [19–21]. Studies of acute lymphoblastic leukemia and non-Hodgkin's lymphoma also demonstrate that *MDM2* over expression is associated with poor survival and aggressive disease [22–24]. Validating animal studies (described below) demonstrate that *Mdm2* gene dosage is critical for oncogene driven lymphomagenesis. Thus deregulation of *MDM2* gene expression likely contributes to the pathogenesis of a wide range of human tumors.

4. *MDM2* functions in normal and malignant cells

Upon genotoxic or cellular stress, increased p53 activity transactivates numerous genes encoding effectors critical in apoptosis and cell cycle arrest [25]. Additionally, p53 activates the *MDM2* gene that encodes a p53 inhibitor forming an autoregulatory feedback loop that tightly controls expression of both p53 and *MDM2* [26]. Multiple *MDM2* activities inhibit p53 function. *MDM2* binds to p53 via an N-terminal domain and directly inhibits the ability of that protein to transactivate its target genes [27]. *MDM2* also directs the export of p53 away from its site of action in the nucleus to the cytoplasm, thanks to a central nuclear export signal [28,29]. Finally, *MDM2* helps to target p53 for degradation through its E3 ubiquitin ligase activity [30–32].

MDM2 also possesses p53 independent, cell-cycle specific functions relevant to tumorigenesis. These include regulation of $p21^{WAF1/CIP1}$ protein