

Tumorigenicity of the MDM2 Oncogene: Beyond p53

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Abstract

The *MDM2* (mouse double minute 2) oncogene is amplified or overexpressed in many human cancers. The expression of *MDM2* is induced by p53, and the MDM2 oncoprotein, as the ubiquitin E3 ligase of p53, functions as a negative regulator of p53. Therefore, inactivation of the MDM2–p53 negative feedback loop has been suggested to be a novel target for cancer therapy. With a second-generation antisense antihuman-*MDM2* oligonucleotide, I have demonstrated anticancer, chemosensitization, and radiosensitization effects, *in vitro* and *in vivo*, of *MDM2* knockdown, in both *p53* wild-type and mutant/null human cancers (*Proc Natl Acad Sci U S A.* 2003,100:11636; *Clin Cancer Res.* 2004,10:1263). Several questions remain unanswered: What are the underlying mechanisms responsible for the p53-independent activities of *MDM2* knockdown? Why are there differences in the accumulation of p53 after *MDM2* knockdown in different cancer cells harboring wildtype *p53*? Is there a better strategy for targeting *MDM2*? The following studies were designed to answer these questions.

Experimental Design:

1. To elucidate the p53-independent activities of MDM2, we used PC3 (*p53* null) cells to screen the potential targets of MDM2, independent of p53.
2. To explain why accumulation of p53 after *MDM2* knockdown in different cancer cells is not same, we hypothesized that, at the posttranslational level, there are unidentified novel regulators of the MDM2-p53 interaction. These molecules may regulate the activity of MDM2. To search for these molecules, we performed yeast two-hybrid assays.
3. To develop better strategies for targeting *MDM2*, we screened more than 50 small molecules for their activities in down-regulating the levels of MDM2 in cells.

Results:

1. We found that, in PC3 cells with *MDM2* knockdown, the level of p21^{Waf1}, another tumor suppressor, is increased. There is direct physical binding between MDM2 and p21^{Waf1}, and this interaction results in destabilization of the p21^{Waf1} protein. In these cells, knockdown of endogenous p21^{Waf1} abrogates the anticancer activity of *MDM2* antisense oligonucleotides (*J Biol Chem.* 2004,279:16000).
2. Also in PC3 cells, the level of E2F1, which has regulatory roles in cell cycle progression and apoptosis, is decreased after *MDM2* knockdown. A physical interaction between MDM2 and E2F1 occurs, resulting in stabilization of the E2F1 protein. In H1299 cells (*p53* null), knockdown of endogenous *E2F1* abrogates the anticancer activity of *MDM2* antisense oligonucleotides (*Oncogene* 2005,24:7238).

3. By use of yeast two-hybrid assay, two novel MDM2 binding molecules have been identified: ribosome protein S7 and proteasome activator PA28 γ . The physical interaction of MDM2 and S7 results in the stabilization of p53 through modulation of the interaction between MDM2 and p53. The stabilized p53 induces apoptosis and cell cycle arrest in human cancer cells (*Oncogene* 2007,26:5029).
4. PA28 γ binds to both MDM2 and p53 and is essential for the MDM2-p53 interaction. Knockdown of PA28 γ in human cancer cells and transgenic mice results in disruption of the MDM2-p53 complex and increase in the level of p53, which induces apoptosis in human cancer cells (*EMBO J.* revision submitted).
5. One of the small molecules screened, genistein, down-regulates MDM2 at the mRNA level. The anticancer effects of genistein are demonstrated through MDM2, *in vitro* and *in vivo* (*Cancer Res.* 2005,65:8200).
6. Curcumin, another small molecule down-regulating MDM2 at the mRNA level, decreases the level of MDM2 through modulation of the PI3K-mTOR-ETS2 pathway. The anticancer effects of curcumin are demonstrated through MDM2, *in vitro* and *in vivo* (*Cancer Res.* 2007,67:1988).

Conclusions:

The tumorigenicity of MDM2 was previously thought to be exclusively through its negative effects on the tumor suppressor p53. Our studies suggest that the expression of MDM2 can be activated, independent of p53, by the PI3K-mTOR-ETS2 pathway, which is coupled to growth factor receptors. In addition to p53, MDM2 also regulates the levels and functions of other proteins, such as p21^{Waf1} and E2F1, resulting in the inhibition of apoptosis and cell proliferation. These activities of MDM2 are modulated by interacting proteins, including S7 and PA28 γ . Thus, our studies shed light on the regulation and functions of MDM2, and provide evidence supporting the concept that MDM2 is a novel target for human cancer therapy and prevention, both dependent and independent of p53. The results indicate that MDM2 inhibitors have a broad spectrum of activity against human cancers, regardless of their p53 status, and provide a basis for the development of novel approaches for human cancer therapy and prevention.