Subject Review

MDM2 and Prognosis

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Abstract

The cellular stress response pathway regulated by the p53 tumor suppressor is critical to the maintenance of genomic integrity and to the prevention of oncogenic transformation. Intracellular levels of p53 are tightly regulated by an autoregulatory feedback loop comprised of p53 and MDM2. It might be predicted that disruption of this loop, either through p53 mutation or overexpression of MDM2, would be a negative prognostic marker for cancer development, likelihood of relapse, or response to therapy. In fact, although MDM2 overexpression is common in cancer, it can be both a positive and a negative predictor of outcome in different tumors, and its significance as a biomarker remains controversial. Data from a number of different tumor types are reviewed for the predictive significance of MDM2 expression, along with evidence for different mechanisms of MDM2 overexpression in these different tumors. In light of the biological complexities underlying the p53-MDM2 loop, it is, perhaps, not surprising that no simple paradigm exists that is generally applicable. Much work remains to be done to elucidate the basic mechanisms underlying the physical interactions between the two proteins, the role of protein modifications in altering those interactions, and also the genetic and transcriptional deregulations by which protein levels are altered in human cancers. Only in this way will truly biologically relevant predictive factors emerge.

Introduction

The p53 tumor suppressor is central to the process by which a cell senses and responds to a variety of potentially oncogenic stresses, and it thereby prevents a damaged and potentially malignant cell from developing into a full-blown cancer. When exposed to stress such as DNA damage, different signal transduction pathways are activated, resulting in modifications of the p53 protein. These stabilize p53, and intracellular levels rise. At the same time, they activate p53 as a transcription factor. It, then, directs stress-specific transcriptional response programs, leading to growth arrest, senescence, or apoptosis (1, 2). Underscoring the critical role of p53 to this process is the observation that it is mutated in approximately half of all cases of cancer.

p53 not only induces these response programs, but it also tightly regulates its own intracellular level through an autoregulatory feedback loop with MDM2[1]. Activated p53 induces the transcription of MDM2, which binds to p53 and inactivates it, and which functions as an ubiquitin E3 ligase to target p53 to the proteosome for destruction (3).

As a negative regulator of p53, it might be predicted that *MDM2* is a proto-oncogene, and that overexpression of MDM2 would be oncogenic by preventing the accumulation of activated p53. Indeed, there is considerable evidence to support this hypothesis. Mice overexpressing Mdm2, for example, develop tumors at an increased rate, in particular, tumors commonly seen in transgenic mice with mutant p53, such as thymic lymphomas and sarcomas (4). In humans, overexpression of MDM2 is common in a variety of different tumor types (5).

In many different tumor types, the loss of p53 is a poor prognostic marker. Likewise, as the functional equivalent of the loss of p53, the prediction is that MDM2 overexpression also correlates with poor prognosis, and can be a useful prognostic tool. The purpose of this review is to examine and summarize data from different human tumors in which it has been attempted to translate this prediction into the clinical realm.

A goal of cancer biology is the identification of biomarkers predictive of disease or therapeutic response. Cancer is a complex disease, however, resulting from the deregulation of diverse interacting pathways. Translational studies, in which it is attempted to link alterations in simple biomarkers with distinct disease phenotypes, must be interpreted with caution. Although overexpression of MDM2 is common in cancer, its value as a prognostic marker remains unclear, depending on tumor type, tissue of origin, and other factors. Indeed, whereas MDM2 overexpression does correlate with poor prognosis in some tumors, a paradox is that it correlates with good prognosis in others.

The major theme of this review is that a simple and reductionist approach to the interpretation of a single biomarker in tumors is unrealistic and often misleading. Several different tumors types will be used as examples to point out the difficulties inherent in attempting to extrapolate interpretations of very complex biological pathways and systems from simple markers. As will also become clear throughout this review, MDM2 itself presents unique challenges that limit its utility at present as a

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[1]The abbreviations used are *MDM2*, human gene and oncogene; MDM2, human protein and isoform; *mdm2*, mouse gene; Mdm2, mouse protein.

prognostic marker in the absence of both better reagents and a more profound appreciation of the biology underlying MDM2 deregulation and its relationship to the p53-MDM2 axis.

Mechanisms of MDM2 Overexpression in Human Cancers

Upstream of exon 1 of MDM2, the P1 promotor site regulates the constitutive expression of the full-length MDM2 mRNA. Within the first intron of MDM2, the p53-responsive P2 promotor site regulates the damage inducible expression of an alternatively spliced mRNA species. MDM2 protein overexpression generally results from gene amplification and the concomitant appearance of double minutes (hence, the name murine double minute). Although protein overexpression in the absence of gene amplification has also been observed, activating mutations have not. This suggests that either: (*a*) MDM2 overexpression can be p53 independent in some cell types; (*b*) MDM2 preferentially binds to and targets for destruction transcriptionally inactive p53; (*c*) MDM2 overexpression is, in some settings, a "readout" for transcriptionally active p53; or (*d*) there are pools within a cell of active and inactive MDM2 that do not directly correlate with overall MDM2 expression but which may reflect different isoforms or modified forms of the protein. Likewise, altered rates of transcription, mRNA stability, enhanced translation, and diminished destruction of the protein all will affect intracellular levels of MDM2.

The relative importance of these different mechanisms of MDM2 overexpression in the absence of gene amplification is not known, nor is it clear how they impact on normal MDM2 function and the p53-MDM2 autoregulatory feedback loop.

Methods

The majority of studies cited in this review use immunohistochemistry (IHC) to ask whether MDM2 is overexpressed in tumors as compared to normal cells, and then to ask whether altered protein levels correlate with a clinical end point. To attempt to link MDM2 overexpression with the disruption of the p53-MDM2 axis, p53 protein status has also been determined in a subset of these studies. Furthermore, to determine whether MDM2 overexpression correlates with the disruption of cell cycle regulatory or apoptotic pathways, the status of proteins involved in these pathways has been determined in other studies. The goal of these studies is not only to identify useful diagnostic and prognostic tools, but also to understand the biology of these tumors by linking mechanistic aberrations to disease.

There are, however, significant caveats to IHC that limit the utility of these studies. In normal cells, for example, p53 protein often cannot be detected, because MDM2 binds to p53 and immediately targets it for degradation. However, by IHC, a nonsense mutation that leads to little or no protein translation is indistinguishable from normal. In contrast, a missense mutation leading to a transcriptionally inactive p53 protein will accumulate within a cell to high levels, because it is unable to transactivate MDM2, and, therefore, cannot be degraded. Both high and low protein levels, therefore, are compatible with inactivating mutations in p53. Because the loss of normal p53 activity in a tumor, however, most often results from a mutation within the p53 open reading frame, and especially within its DNA binding domain, the p53 mutation status of a tumor can be confirmed using standard molecular techniques, such as single-strand conformation polymorphism (SSCP)-sequencing of tumor DNA. Hence, it is straightforward to identify tumors with mutant p53, and to correlate outcome with loss of p53.

With respect to MDM2, functional alterations rarely result from mutations, and so, analysis is more complex. There are a number of different protein isoforms distinguishable by Western blot analysis, the relative proportion of which can be altered in tumors, but all of which cross-react with available antibodies. Hence, they cannot be readily differentiated by IHC. In addition, whether these isoforms are functional, dominant-negative forms, or inactive breakdown products of the full-length protein is unknown. Furthermore, as an E3 ligase, MDM2 must shuttle between the nucleus and the cytoplasm to bind and then degrade p53. Subcellular localization of MDM2 to either the nucleus or the cytoplasm, therefore, reflects the ability of the protein to effectively shuttle, and may be an indirect index of function. In addition, both MDM2 and p53 are modified by phosphorylation, as well as by other mechanisms. Differential modification of these molecules can both promote and prevent physical interaction between the proteins, and therefore, regulate the ability of MDM2 to inactivate and degrade p53. Immunostaining for total protein within tumors does not take into account the impact of these modifications on function. Indeed, the critical functional modifications remain poorly understood for both molecules, and it remains to be seen whether alterations in total protein levels within tumors correlate with alterations in function.

A final limitation is that even the definition of "overexpressed" for MDM2, as well as other proteins, is not standardized. Within a tumor, there is heterogeneity in immunostaining; the appropriate cutoff for percent cells positive that actually correlates with overexpression, therefore, remains controversial. To further obscure the meaning of overexpression, there are no commonly accepted criteria for positive immunostaining, and the role of tissue-specific factors that may alter basal protein levels is unknown. Immunopositivity, then, is a subjective decision, and may reflect investigator bias, sample bias, or even staining artifacts. In light of the increasing use of high-density tissue microarrays to screen large numbers of tumors in parallel, the size of each embedded tumor tissue block in the array is very small, and the possibility of inadvertent sample selection bias is increased.

In summary, interpretation of MDM2 expression in tumors is limited by: (*a*) multiple protein isoforms and protein modifications; (*b*) antibody specificity and cross-reactivity; (*c*) the absence of a standard definition for overexpression; (*d*) the lack of distinction between cellular *versus* nuclear or cytoplasmic staining; (*e*) interpretation bias; (*f*) sample bias; and (*g*) other factors.

MDM2 and Prognosis in Human Cancers

Sarcomas

The initial hypothesis was that in tumor types in which alterations of p53 were common, an alternative mechanism for the inactivation of the p53 tumor suppressor pathway was the

Table 1. MDM2 Alterations in STS

Cohort	MDM2 Overexpression	Mechanism	p53 Overexpression	MDM2 and Survival	Reference
24	8/24	Gene amplification	8/24	N/A	6
211	76/211	N/A	56/211	MDM2+/p53+: poor	7
198				MDM2−/p53−>	8, 9
86				MDM2−/p53+> MDM2+/p53−> MDM2+/p53+	
67	19/67	Gene amplification		MDM2+: good	11

overexpression of MDM2 (Table 1). Loss of p53 is a common event in soft tissue sarcomas (STS). In a cohort of 24 human STS (11 fibrous histiocytomas and 13 liposarcomas), 8 tumors had alterations in p53, while a different set of 8 tumors had *MDM2* gene amplifications with elevated levels of nuclear MDM2 staining by IHC (Table 1; 6). This suggested, therefore, that these two events were, indeed, alternate mechanisms for the inactivation of the same pathway. In a larger cohort of 211 STS, however, it was observed by Cordon-Cardo *et al.* (7) that while 76 of 207 tumors overexpressed MDM2 and 56 of 211 overexpressed p53 (indicative of the accumulation of functionally inactive mutant p53), both proteins were overexpressed in 22 cases. Surprisingly, this group had the worst prognosis, suggesting a p53-independent oncogenic role for MDM2. This was again observed in a cohort of 198 STS of known p53 status stained by IHC for MDM2, and then stratified by staining into prognostic groups by Wurl and colleagues. It was found that patients with negative staining for both p53 and MDM2 had the best prognosis, an observation consistent with the preservation of the normal p53-MDM2 axis in tumors from these patients; those with either biomarker positive had intermediate prognosis; and those positive for both proteins had the worst outcome (RR = 4.63, $P = 0.00001$; 8). A later study by the same group of 86 STS of the extremities adjusted for clinico-pathological characteristics refined this hierarchical risk stratification. Again, p53/MDM2 negative tumors had the best prognosis; then MDM2−/p53+; then MDM2+/p53−; then MDM2+/p53+, with an RR = 18.77, $P = 0.006$ (9). The fact that Wurl *et al.* observed that MDM2 overexpression was worse prognostically than p53 overexpression again suggested a p53-independent oncogenic role for MDM2. Interestingly, in another study, this same group observed that overexpression of both MDM2 and the retinoblastoma (RB) tumor suppressor also conferred a worse prognosis, with an RR = 3.30, $P = 0.002$ (10). The normal function of RB is as a negative regulator of cell proliferation. The finding that the concomitant overexpression of MDM2 and either p53 or RB confers worse prognosis suggests that one p53-independent oncogenic function of MDM2 may be to perturb the normal antiproliferative function of the RB tumor suppressor. Indeed, Ki-67 positivity or proliferating cell nuclear antigen (PCNA) overexpression, both markers of cell proliferation, are often observed in these tumors and are associated with poor outcome.

While these data point to the overexpression of MDM2 in STS as a negative predictor, it was found in one recent study that *MDM2* gene amplification was more frequently noted in grade I tumors than in higher grade tumors and that it correlated with prolonged survival in STS, with a mean survival of 87 months, as compared to 40 months in patients whose tumors lacked the gene amplification ($P = 0.02$; 11). Therefore, although there is considerable evidence that MDM2 overexpression and gene amplification are common within this single subtype of histologically similar sarcomas, there are significant discrepancies as to the clinical significance of this observation. The studies by Wurl and colleagues, in which there are reproducible inter-study findings and correlations, underscore the importance of consistency, both in methodology and in investigator scoring in studies attempting to identify tumor biomarkers by IHC.

STS as a group represent tumors diverse in histopathological presentation and biological behavior. STS in adults are relatively slow-growing and well-differentiated tumors. Pediatric sarcomas, in contrast, are generally aggressive and highly anaplastic. In two pediatric sarcomas, osteogenic sarcoma and rhabdomyosarcoma, *MDM2* gene amplification is a common occurrence (12). Waber *et al.* (13) surveyed a series of 71 morphologically similar pediatric sarcomas of differing histological origins, and did not identify a single tumor in which the *MDM2* gene was amplified. They did not, however, ask whether the protein was overexpressed in this cohort. In another cohort of 20 pediatric rhabdomyosarcomas, MDM2 was amplified in DNA isolated from tumors from only 2 patients (14). Strikingly, a complete response to therapy was obtained in both of these patients.

One explanation for these differing observations is that the biology of a tumor is the result not only of the deregulation of critical pathways, such as the p53 tumor suppressor pathway, but also the tissue of origin. Distinct tissue types differ with regard to gene expression patterns, proliferative indices, and specific stresses to which they are exposed and susceptible. It suggests that there are tissue-specific, differentiation-specific, or growth-specific pressures for pathway inactivation that cannot be generalized.

Even within a single tumor and tissue type, data from different groups are difficult to reconcile, suggesting again that different methodologies may underlie different conclusions. Antonescu *et al.* (15) studied a cohort of 49 patients with localized synovial sarcoma (SS) of the extremities. They observed by IHC that although p53 overexpression was a relatively uncommon event in this tumor (16% of those studied), it was an independent predictor of tumor relapse ($P = 0.001$). MDM2 overexpression was very common (59%) but was not of prognostic value ($P = 0.6$). In light of the association between MDM2 overexpression, Ki-67 positivity,

and poor prognosis in STS, it is of interest to note that Ki-67 immunoreactivity was observed in 59% of the SS tested, and that it was an independent predictor of poor prognosis ($P = 0.002$). However, there was no correlation between MDM2 overexpression and Ki-67 immunoreactivity (15), suggesting either MDM2-independent pro-proliferative factors in SS, or methodological differences in the analysis of the different tumors between the two groups.

Oda *et al.* (16) studied a similar cohort of 49 patients with SS, looking not only at tumor immunoreactivity, but also at p53 mutation status by PCR-SSCP. In this study, loss of p53 was found to be far more common and MDM2 overexpression far less common than reported by Antonescu *et al.* (15). They observed that p53 was mutant in 9 of 49 tumors and overexpressed in 11 of 49 tumors, but that only 3 of these had concomitant p53 mutations by PCR-SSCP. Nineteen of 49 tumors overexpressed MDM2, almost all by gene amplification.

There was a significant positive correlation noted between p53 immunoreactivity and MDM2 overexpression, suggesting either that there is a common mechanism leading to both phenotypes, or that there is pressure in SS to disrupt both proteins, producing a negative cooperative effect. In contrast to the findings by Antonescu *et al.* (15), and despite the frequency with which the p53-MDM2 loop was found to be disrupted in this study, Oda *et al.* (16) did not observe any correlation between altered p53 or MDM2 status, and outcome.

Gliomas

Malignant gliomas represent another tumor type in which loss of p53 function is common, with mutations of p53 found in roughly one-third of all glioblastomas (17). Occurring in about 10% of gliomas, amplification of MDM2 is also a common event in these tumors, representing the second most common amplification, after that of epidermal growth factor receptor (18). In gliomas, gene amplification is the major mechanism by which MDM2 overexpression occurs (Table 2). In gliomas, the frequency of MDM2 amplification occurs in a different subgroup of patients than does loss of p53, suggesting that in this tumor type, these are alternate mechanisms of inactivating the p53 pathway (19). Despite the evidence that both loss of p53 and MDM2 overexpression are oncogenic by disrupting the p53-MDM2 loop, the prognostic implications of p53 mutation and MDM2 overexpression differ markedly, although this is controversial. In one study of 61 patients with central nervous system gliomas, Korkolopoulou *et al.* (20) found that whereas grade and high proliferative indices were associated with reduced survival, immunohistochemical expression of MDM2 and p53 were both independent predictors of improved overall survival. In contrast, in another study on a cohort of 107 patients with high-grade gliomas, it was observed that overexpression of MDM2 was significantly correlated with shortened survival ($P = 0.02$; 21). This was also observed in a study of 75 patients with glioblastomas, in which MDM2 amplification and p53 mutation status were correlated with survival. One-third of tumors had p53 mutations, while MDM2 was amplified in 13% of the tumors. In no case did the two events occur in the same tumor. Surprisingly, it was observed that p53 mutations occurred in tumors from patients significantly younger than those in whom MDM2 was overexpressed. Furthermore, while loss of p53 was associated with prolonged survival by Kaplan-Meier analysis, amplification of MDM2 was found to be an independent predictor of bad outcome (17). However, other recent studies have cast some doubt on this correlation between MDM2 amplification and poor prognosis (22, 23). If amplification of MDM2 is associated with shortened survival, it might be predicted that it would be frequent in recurrent high-grade gliomas. When studied, however, there was no significant difference in the frequency of MDM2 amplification in primary *versus* recurrent tumors (24).

Hematological Malignancies

In contrast to sarcomas, gliomas, and lymphomas, loss of p53 is a very rare event in pediatric acute lymphoblastic leukemia. One possible explanation is that there are other mechanisms for the functional inactivation of p53 that predominate in these tumors. In sarcomas, MDM2 is overexpressed in about one-third of all cases. In a cohort of 48 children with leukemia, Bueso-Ramos *et al.* (25) did not detect *MDM2* gene amplification at all; in contrast, MDM2 mRNA overexpression was observed in 53% of cases, in some with levels approaching 50-fold that were found in normal bone marrow cells. Furthermore, MDM2 overexpression was predominantly associated with leukemias with unfavorable chromosomal markers (25). In a larger cohort of 135 cases of leukemia, no cases with p53 mutations were identified, nor were there any leukemias in which the *MDM2* gene was amplified. However, of 9 ALL cases studied, MDM2 mRNA was overexpressed in 3, of which all were associated with poor response to therapy (26).

Specifically with respect to pediatric ALL, in an analysis of 10 diagnosis bone marrows from children who either did not achieve remission or who relapsed within 6 months of achieving remission, p53 mutations were seen in 3 children, with MDM2 overexpression observed in 4 children, all at the mRNA level (27). On the basis of these observations, a

Table 2. MDM2 Overexpression and Survival in Selected Cancers

Cancer	MDM2 Overexpression	Mechanism	p53 Overexpression	MDM2 and Survival	Reference
Glioma	Common	Gene amplification	Common	MDM2+: poor	17, 20, 21
ALL	Common	Transcription/Translation	Rare	MDM2+: poor	25–31
Melanoma	Common	Transcription/Translation	Rare	MDM2+: good	36, 37
NSCLC	Common	Amplification: Rare Transcription/Translation		MDM2+: good	38, 39
Breast	Common	Transcription		MDM2+: good (ERα+)	41, 42

Note: NSCLC, non-small-cell lung cancer.

subsequent matched retrospective case-controlled study was undertaken to ask whether altered p53 function in diagnosis marrow samples from children with ALL treated on the same protocol correlated with early treatment failure. Of 17 children with good outcome, a mutation in p53 was detected by SSCP/DNA sequence analysis in only 1 child, and no children had altered levels of MDM2 protein, by Western blot analysis. In contrast, 2 of 17 children in the poor outcome group had mutations in p53, while 5 overexpressed MDM2 protein >10-fold, compared to the good outcome group (28). This study demonstrated that alterations in p53 pathway function were significantly more common in children with poor outcome than in those with good outcome ($P = 0.036$). Even among poor responders, children in whom MDM2 protein was overexpressed >10-fold over normal by Western blot analysis in bone marrow mononuclear cells at the time of relapse comprised a group at particularly high risk. The median length of time of the first complete remission in this group was 20.5 months, as compared to 41 months for non-overexpressors ($P < 0.01$). *In vitro*, cells from these children were highly resistant to doxorubicin ($P < 0.005$; 29).

These data indicating that MDM2 protein overexpression was a marker of poor prognosis were confirmed immunohistochemically in several studies done in Sweden on a genetically homogeneous population of children with ALL treated on the same protocols (30). They observed that while p53 expression was rare in children who achieved durable remission at the time of diagnosis (4 of 30), it was observed in bone marrow mononuclear cells in 8 of 15 children admitted for bone marrow transplant (BMT) ($P = 0.014$). MDM2 expression was noted at diagnosis in cells from 4 of 30 children in the non-relapse groups, but in 10 of 15 children at the time of BMT ($P = 0.0011$). Of the 15 children in the BMT group, cells from 7 children at the time of diagnosis were available. Of these, p53 expression was more common than in the relapse-free group ($P = 0.054$), as was MDM2 expression ($P = 0.0098$; 31). Hence, in pediatric ALL, deregulation of the p53 pathway is rarely achieved through inactivating mutations in p53. Rather, elevated levels of MDM2 mRNA by an unknown mechanism results in protein overexpression, which is especially correlated with poor prognosis and shortened survival.

In transgenic mice with mutant p53, thymic lymphomas are the most commonly occurring tumors. Therefore, it might be predicted that deregulation of the p53 pathway would be a feature of lymphoma. In a Danish study of 188 non-Hodgkin's lymphoma (NHL), it was observed that overexpression of MDM2 occurred in 22% of the tumors and was particularly associated with high-grade/aggressive disease ($P < 0.0001$) and shortened survival, independent of histological type (32). Furthermore, in sequential studies on patients with relapsing NHL with or without transformation, it was observed that while no indolent NHL overexpressed MDM2, it was common in relapsed and transformed disease (33–35).

Melanoma

Malignant melanoma is another tumor type in which p53 mutations are rare. In a cohort of 172 patients with cutaneous melanoma at different stages along the pathway of melanocyte transformation, it was observed that MDM2 overexpression by IHC was present in <50% of cases of primary invasive disease, regardless of the depth of invasion, as well as in metastatic disease. In only one case was *MDM2* gene amplification detected, either by Southern blot analysis or by FISH, suggesting that, as in pediatric ALL, the mechanism driving overexpression was either transcriptional or posttranscriptional (36). Surprisingly, however, when MDM2 was correlated with outcome in a cohort of 134 patients followed at a single institution over 10 years, it was found that MDM2 overexpression was an independent predictor of survival (RR = 0.55, $P = 0.027$; 37).

Carcinomas

In NSCLC, immunohistochemical detection of MDM2 expression was identified as a favorable prognostic marker in a cohort of 201 patients for the subset of 110 patients without concomitant expression of p53 ($P = 0.037$). As in melanoma, the frequency of *MDM2* gene amplification is low (2 of 30; 38). However, in a separate study, it was observed that the presence of MDM2 mRNA in NSCLC, as detected by reverse transcription-PCR, was an independent favorable prognostic factor for survival by Kaplan-Meier analysis (39).

In breast cancer, it was observed that MDM2 mRNA was overexpressed relative to normal breast tissue and correlated with protein overexpression by IHC in 24 of 33 tumors tested. Gene amplification was not observed (40). Multiple MDM2 mRNA species not found in normal breast tissue were identified, and the presence of these species correlated with worse outcome (41). When MDM2 overexpression was correlated to estrogen receptor α (ERα) status, it was observed that MDM2 overexpression occurred only in ERα-positive tumors, but not the MDM2 mRNA splice variants, and that in ERα-positive tumors, MDM2 overexpression conferred a favorable prognosis (42). In tissue culture model systems, it was recently shown that p53-independent transactivation of MDM2 in breast cancer cell lines was a function of ERα status and could only occur in cell lines that were ERα-positive, thereby leading to the hypothesis that p53-independent pathways can modulate the p53 pathway by altering levels of MDM2 (43).

Conclusions and Future Directions

In summary, in sarcomas and gliomas, MDM2 overexpression commonly resulted from gene amplification, and, in most studies, correlated with poor prognosis. In pediatric ALL, overexpression of MDM2 occurred in the absence of gene amplification but was still predictive of bad prognosis. In melanoma, MDM2 overexpression also occurs in the absence of amplification but confers a favorable prognosis. In NSCLC, MDM2 overexpression also confers good prognosis in the absence of gene amplification, but is correlated instead with elevated levels of MDM2 mRNA, which in pediatric ALL, correlated with poor outcome. In breast cancer, the survival advantage of MDM2 overexpression is evident only in the subset of patients that are ERα, and appears to be a function of p53-independent transactivation of MDM2.

In addition to the methodological limitations of immunohistochemical analysis of tumor markers discussed earlier

in this review, there are many levels of biological complexity that make it difficult to interpret these data and to devise a model to explain the functional significance of MDM2 overexpression in various tumors. Without a more complete understanding of underlying mechanisms of MDM2 overexpression, as well as of the various pathways regulating MDM2 level and activity and regulated by MDM2, the prognostic value of MDM2 as a marker remains limited.

If the loss of p53 and the overexpression of MDM2 are alternate mechanisms to disrupt p53 tumor suppressor pathway function, it is difficult to understand why the loss of p53 and the overexpression of MDM2 in tumors do not in all cases correlate with poor prognosis. Furthermore, it is unclear why, within a single tumor type, loss of p53 can be associated with one clinical outcome, while MDM2 overexpression is associated with a different outcome.

The observation that p53 is overexpressed in some tumor types can be explained by the hypothesis that it represents transcriptionally inactive p53 that cannot induce MDM2, and therefore, cannot be degraded. Because p53 transactivates MDM2, the observation in a single tumor that both p53 and MDM2 can be overexpressed was unexpected, as was the finding that coexpression often confers poor prognosis. One explanation would be that MDM2 does, indeed, have in some cell types an additional p53-independent antitumor activity, for example, as a regulator of cell proliferation. In this model, loss of p53 would not relieve selective pressure within a cancer cell to overexpress MDM2. The concomitant overexpression of both proteins would be predictive of worse prognosis than either singly. In addition, the relative importance of p53-dependent and p53-independent functions of MDM2 in different tissues may underlie the different prognostic implications of MDM2 overexpression in different tissues.

Specifically with respect to MDM2, the correlation between detectable level and function is not simple. First, the significance of different mechanisms of overexpression is not known. Why, for example, does gene amplification predominate in some cell types but not in others? Why do similar mechanisms of overexpression in different cell types correlate with different clinical outcomes? Second, a number of different mRNA species are found in both normal cells and in tumor cells, some of which are unique to tumors. In addition, available antibodies detect several different protein isoforms, some of which are tumor specific, the functions of which are unknown. Whether these different mRNA species and protein forms represent functionally altered MDM2 remains unclear. Finally, MDM2 is heavily modified by phosphorylation. It is thought that these modifications both positively and negatively regulate MDM2 binding to p53, thereby altering its ability to target p53 for destruction. Hence, it is unclear whether increased intracellular levels of MDM2 correspond to increased levels of functional MDM2. In addition, it is also unclear whether deregulation of other pathways that may regulate MDM2 levels in a p53-independent manner also alter the p53 pathway.

Recently, two proteins, p63 and p73, have been identified that are members of the p53 gene family. A number of different isoforms of both of these proteins have been identified, both transcriptionally active and transcriptionally inactive (44–47). It has also been demonstrated that both proteins can induce apoptosis and cell cycle arrest (48). Different isoforms, however, differentially transactivate p53 target genes (46–48). In contrast to p53, p63 can bind to MDM2, but it is unclear whether MDM2 targets p63 for degradation, and may, in fact, increase its transcriptional activity (49, 50). p73 can transactivate MDM2 and bind to the MDM2 protein, but it is also not targeted for degradation by MDM2 (51). Degradation of p73 is instead mediated by the deltaN-isoform, which can be induced both by the TA-isoform and also by p53 (44, 45). Hence, a complex network of interdependent regulation exists among these three closely related proteins. It is clear that alterations of p63 occur in a number of carcinomas, including bladder cancer (52) and nasopharyngeal carcinoma (53). Likewise, alterations in p73 have been observed in neuroblastoma (54), melanoma (55), and in gliomas (56). The significance of these alterations remains to be seen. It is unclear whether, for example, they are primary events, or occur as a result of a primary disruption in the p53 pathway. Given that both p63 and p73 can interact with and regulate levels of MDM2, it may be that some of the apparent discrepancies in the predictive significance of MDM2 in different tumor types may result from primary alterations of p63 or p73.

To add even further complexity to this emerging and expanding regulatory network, MDMX is an MDM2-related protein that regulates intracellular levels of the p53 protein in an MDM2-independent manner (57). Amplification and overexpression of MDMX has recently been observed in a subset of patients without p53 mutation or MDM2 amplification (58). The clinical and prognostic significance of this observation remains unclear.

Unlike tissue culture or even murine models, humans are wonderfully complex biological systems. Clearly, much work remains to be done to understand the clinical relevance of MDM2 in human cancer. The challenge for the future is to develop high-throughput platforms that allow for the simultaneous analysis of many putative biomarkers, representing many pathways, at the DNA, RNA, and protein levels. Ultimately, the sum of these data will provide the insights by which mechanism can be correlated with cancer phenotype and clinical outcome, and the power of molecular markers as prognostic tools will be evident.

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