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SURVEY AND SUMMARY

The *MDM2* gene amplification database

Jamil Momand*, David Jung[1], Sharon Wilczynski[2] and Joyce Niland[1]

Department of Cell and Tumor Biology, Beckman Research Institute and Departments of [1]Biomedical Informatics and [2]Pathology, City of Hope National Medical Center, 1450 East Duarte Road, Duarte, CA 91010-3000, USA

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ABSTRACT

The *p53* tumor suppressor gene is inactivated in human tumors by several distinct mechanisms. The best characterized inactivation mechanisms are: (i) gene mutation; (ii) p53 protein association with viral proteins; (iii) p53 protein association with the MDM2 cellular oncoprotein. The *MDM2* gene has been shown to be abnormally up-regulated in human tumors and tumor cell lines by gene amplification, increased transcript levels and enhanced translation. This communication presents a brief review of the spectrum of *MDM2* abnormalities in human tumors and compares the tissue distribution of *MDM2* amplification and *p53* mutation frequencies. In this study, 3889 samples from tumors or xenografts from 28 tumor types were examined for *MDM2* amplification from previously published sources. The overall frequency of *MDM2* amplification in these human tumors was 7%. Gene amplification was observed in 19 tumor types, with the highest frequency observed in soft tissue tumors (20%), osteosarcomas (16%) and esophageal carcinomas (13%). Tumors which showed a higher incidence of *MDM2* amplification than *p53* mutation were soft tissue tumors, testicular germ cell cancers and neuroblastomas. Data from studies where both *MDM2* amplification and *p53* mutations were analyzed within the same samples showed that mutations in these two genes do not generally occur within the same tumor. In these studies, 29 out of a total of 33 *MDM2* amplification-positive tumors had wild-type p53. We hypothesize that heretofore uncharacterized carcinogens favor *MDM2* amplification over p53 mutations in certain tumor types. A database listing the *MDM2* gene amplifications is available on the World Wide Web at http://www.infosci.coh.org/mdm2 . Charts of *MDM2* amplification frequencies and comparisons with *p53* genetic alterations are also available at this Web site.

INTRODUCTION

The *p53* tumor suppressor gene is the most frequently inactivated gene in human malignancies analyzed to date. In ~40% of the tumor samples *p53* is inactivated by mutations within the coding region of the open reading frame (1). In most tumors, a point mutation usually occurs in one allele and the second allele is deleted. In some instances, however, the *p53* gene is wild-type but its protein product is inactivated by viral and cellular oncogene products. The prevalence of these alternative mechanisms of *p53* inactivation is not yet completely known. One of these alternative mechanisms is through overexpression of the cellular oncogene *MDM2* (2,3).

MDM2 was originally cloned from amplified DNA obtained from a spontaneously transformed murine cell line (4,5). Analysis of the putative MDM2 protein sequence showed that it codes for a 483 amino acid residue protein with a zinc-binding RING finger motif (6). The MDM2 protein is phosphorylated on serine residues and its human homolog is located on chromosome 12q13–14 (3). Several recent reviews on general MDM2 function have been published (7–10) and electronic information, including links to its gene structure, can be found on the World Wide Web site maintained by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?164785).

Due to the rapid pace of *MDM2* research and its clear function in down-regulating p53 activity, we have decided to determine, from original peer-reviewed sources, the frequency of *MDM2* gene amplification events in different types of tumors. Such data could help in several ways, such as selecting tissue types to conduct *MDM2* research, choosing tumors to test new pharmaceuticals that exploit the MDM2–p53 interaction and seeking a greater understanding of carcinogens that initiate gene amplification.

FREQUENCY OF *MDM2* AMPLIFICATION

To date, 3889 tumor tissue samples have been examined for *MDM2* amplifications (Table 1). (For the purposes of this review, the term tumor will be used to signify both benign and malignant growths.) A compilation of these data shows that the overall frequency of *MDM2* amplification is 7%. The highest frequency is observed in soft tissue tumors (20%), which includes Ewing's sarcoma, leiomyosarcomas, lipomas, liposarcomas, malignant fibrous histiocytomas, malignant Schwannomas and other sarcomas such as rhabdomyosarcomas. Osteosarcomas have the second highest frequency of *MDM2* gene amplification (16%). At the other end of the spectrum, several tumors show no *MDM2* amplification, including Wilms' tumors, leukemias, lymphomas, hepatoblastomas and pancreatic carcinomas. Amplification ranges between 2- and 10-fold. The most common technique for detecting *MDM2* amplifications was Southern blotting, although quantitative PCR amplification was employed in a few studies (11,12).

*To whom correspondence should be addressed. Tel: +1 626 301 8878; Fax: +1 626 301 8972; Email: jmomand@coh.org

Table 1. Summary of *MDM2* gene amplification frequencies from 28 human tumors

Tumor type	*MDM2* amplification (n^a) (%)	References
Brain tumors	6.7 (239)	57–60
Astrocytomas	8.1 (37)	57,60
Glioblastomas	6.8 (191)	57,58,60
Medulloblastomas	0 (8)	59
Other	0 (3)	60
Breast carcinomas	5.9 (1774)	61–65
Cervical carcinomas	1.1 (88)	19,66
Esophageal carcinomas	13 (96)	14,67
Leukemias/lymphomas	0 (304)	68–70
Hepatoblastomas	0 (38)	71
Lung	5.7 (88)	72–74
Lung cancers (NSCLC)	6.0 (83)	72,74
Lung (not specified)	0 (5)	73
Nasopharyngeal carcinomas	2.1 (46)	75
Neuroblastoma	2.0 (51)	76–78
Osteosarcomas	16 (207)	3,79–82
Ovarian carcinomas	3.1 (190)	64,83
Pancreatic carcinomas	0 (25)	84
Soft tissue tumors	20 (479)	3,36,76,79,80,85–90
Ewing's sarcomas	10 (30)	85
Leiomyosarcomas	0 (46)	79,86,88
Lipomas (benign)	30 (43)	80,86
Liposarcomas	29 (87)	3,79,80,87,89
Malignant fibrous histiocytomas	21 (163)	3,79,80,86,90
Malignant Schwannomas	19 (16)	79
Sarcomas (non-specific)[b]	13 (85)	36,76
Various[c]	33 (9)	76,79,86
Testicular tumors	4.6 (64)	91,92
Thyroid carcinomas	0 (22)	93
Urothelial carcinomas	2.2 (137)	94,95
Wilms' tumors	0 (40)	76

Total number of tumor samples analyzed was 3889 and the average *MDM2* gene amplification frequency was 7.2%.
[a]Number of samples analyzed.
[b]Sarcomas of soft tissue origin that were not specified.
[c]Soft tissue tumors that did not fall into the listed classes. The number of samples was less than five in any individual class.

COMPARISON OF *p53* GENETIC ALTERATIONS AND *MDM2* AMPLIFICATION FREQUENCIES

Since p53 and MDM2 are directly antagonistic, we hypothesized that *p53* mutations and *MDM2* amplification would tend not to occur in the same tumor samples. To test this we compared the frequency of *p53* genetic alterations and *MDM2* amplification for each tumor type (Fig. 1). The *p53* mutation data is the sum of the missense mutations, mutations in introns affecting splicing, and small frameshift mutations (1,13). Most *p53* mutation data were obtained from reviews (1) and the Web site, http://perso.curie.fr/Thierry.Soussi , maintained by Thierry Soussi (13). However, for soft tissue tumors and osteosarcomas, original publications were used as the source of *p53* mutation frequency data.

Analyses of primary tumor samples show that *p53* mutation and *MDM2* amplification do not generally occur within the same tumor sample (Table 2). Since a *p53* mutation and a *MDM2* gene amplification both prevent p53 function, one would expect a negative association between these two outcomes. If one considers only tumor types where either *p53* mutations or *MDM2* amplification have been observed, then, out of a total of 93 such tumors, 60 had *p53* mutations, 33 had *MDM2* amplification and four had both *p53* and *MDM2* genetic alterations.

The Mantel–Haenszel χ^2 test was used to examine the association between *p53* mutations and *MDM2* amplification, stratifying by tumor type. Significance was set at an α level of 0.05 and the test was performed two-sided. The Breslow–Day test for homogeneity was used to compare odds ratios across strata. Because there was no significant difference in odds ratios across tumor types ($P = 0.40$), a combined odds ratio over all strata and the 95% confidence interval were calculated using the Mantel–Haenszel logit method. There was a statistically significant negative association between the occurrence of *p53* mutations and *MDM2* amplification ($P = 0.038$). The odds of a *p53* mutation occurring if *MDM2* amplification was present was less than a third of that for patients with no amplification present (odds ratio 0.30, 95% confidence interval [0.09, 0.93]).

It is possible that the four esophageal carcinomas reported as having both genetic alterations was the result of a random distribution of mutations (14). Another possibility is that these tumors were heterogeneous in their genetic makeup (i.e. some cells with wild-type *p53*/*MDM2* amplification, other cells with mutant *p53*/normal *MDM2*). Finally, it is possible that *MDM2* may play a p53-independent role in tumor formation. Overall, however, the data suggest that *p53* mutation and *MDM2* amplification tend to be mutually exclusive events and that inactivation of wild-type p53 is the chief responsibility of *MDM2* amplification.

Since p53 and MDM2 lie in the same signaling pathway, one would expect that in tumor types in which the *p53* mutation frequency is low, a higher frequency of *MDM2* amplification would be observed. Figure 1 shows that the *MDM2* amplification frequency is higher than the *p53* mutation frequency in only three tumor types: soft tissue tumors, testicular cancers and neuroblastomas. In these tumors, *MDM2* amplification frequencies were 20, 4 and 2% respectively, whereas *p53* genetic abnormalities occurred in 14, 0 and 1% of these tumors. Overall, p53 does not appear to be inactivated by either *p53* mutation or *MDM2* amplification in testicular cancers or neuroblastomas. The reason for preferred *p53* inactivation through *MDM2* amplification in soft tissue tumors is unclear. One may speculate that the types of mutagens that these tissues are exposed to may predispose them to gene amplification events, rather than deletions and point mutations.

Table 2. Simultaneous analysis of *MDM2* amplification and *p53* genetic alterations suggests that mutations at each locus tend to be mutually exclusive

Tumor type	n[a]	*p53* mutation	*p53* wild-type	*MDM2* amplification	*MDM2* amplification + *p53* mutation[b]	Reference
Osteosarcomas, soft tissue tumors	94	10	84	10	0	79
Esophageal tumors	72	29	43	13	4	14
Urothelial tumors	50	17	33	2	0	94
Liposarcomas	13	4	9[c]	8	0	89
Total	229	60	169	33	4	

[a]Total number of tumors in study.
[b]Statistically significant negative association between *p53* mutation and *MDM2* amplification ($P = 0.038$, Mantel–Haenszel χ^2 test).
[c]Includes one silent mutation.

A corollary to the above scenario is that in tumors with extremely high *p53* mutation frequencies, *MDM2* amplification may be suppressed. Two cancers with high *p53* mutation frequencies are lung and urothelial cancers, where the percentage of *p53* genetic alterations is 70 and 61% respectively (Fig. 1), while the frequency of *MDM2* amplifications is only 6 and 2.2% respectively. These gene amplification frequencies are not drastically lower than the average *MDM2* amplification frequency (7%), but the relative genetic alteration frequencies do indicate that the *p53* pathway is preferentially inactivated by mutations within *p53* in these tumor types. Indeed, known tobacco carcinogens, such as benzo[*a*]pyrene, have been shown to form diol epoxide adducts at lung cancer mutation hotspots within the *p53* gene (15).

The fact that several tumors have a significant number of samples where both *p53* and *MDM2* are not mutated (i.e. neuroblastomas, hematological malignancies, testicular cancer) leads to the obvious possibility that p53 may be inactivated by other, as yet undetermined, mechanisms. This possibility is clearly borne out in cervical cancers. In uterine cervical carcinomas 90% of patients are infected with oncogenic subtypes of the human papilloma virus (HPV) (16). The *E6* oncogene of this virus expresses a product that binds p53 and leads to its degradation (17). Strong data previously showed that in oncogenic HPV-positive cancers, *p53* mutations are observed in <4% of the samples tested (1,18). In this cancer type one would expect the frequency of *p53* genetic alterations and *MDM2* amplification to also be low. This may be the case. In cervical cancer, the frequency of *p53* genetic abnormalities is only 7% and the frequency of *MDM2* amplification is 1%. However, in one of the two *MDM2* amplification studies conducted on cervical cancers, only HPV-negative samples were tested, indicating that the overall frequency of *MDM2* amplification (in HPV^+ and HPV^- samples) is <1% for this cancer (19). Notwithstanding this caveat, the data suggests that p53 can be inactivated by a variety of separate mechanisms.

HIGH INCIDENCE OF *MDM2* AMPLIFICATION IN SOFT TISSUE TUMORS

Apparent predisposition of soft tissue tumors to *MDM2* amplification warrants a closer look at these tumors. Soft tissue tumors are derived from smooth and striated muscle, fat, fibrous tissue, blood vessels and the peripheral nervous system (20). Soft tissue sarcomas are prevalent in a rare familial cancer syndrome called Li–Fraumeni syndrome, or LFS (21). Approximately 50% of LFS patients inherit one mutant allele of *p53* (22–24). The second *p53* allele is sometimes deleted in tumors of these patients, in accordance with the classical two-hit hypothesis first put forward by Knudson (25). However, only in ~50% of tumors of LFS patients is the second *p53* allele lost (26). In patients where mutant *p53* is inherited it would be of interest to determine whether *MDM2* amplification or its overexpression can substitute for loss of the second *p53* allele. In the LFS patients where germline *p53* mutations are not detected, it might be prudent to investigate *MDM2* gene amplification as well. However, in one such LFS family it has been shown, by linkage analysis, that the inherited defect does not map to the chromosomal location where *MDM2* resides (27). Soft tissue sarcomas also arise as secondary tumors in survivors of familial retinoblastomas (20) and breast cancer radiotherapy. It is unclear if *MDM2* plays a role in these secondary tumors.

OTHER MECHANISMS OF MDM2 OVEREXPRESSION IN HUMAN TUMORS

MDM2 may be up-regulated by mechanisms other than *MDM2* amplification, including enhanced translation and gene translocation, although whether these events occur in human tumors is unknown (28,29). *MDM2* transcript levels have been shown to be relatively high in several tumors, for example, leukemias and lymphomas, with no gene amplification (30,31). If *MDM2* is overexpressed through another abnormal mechanism, it would suggest that gene amplification analysis leads to an artificially low frequency of *MDM2* involvement in human tumors. A simple model is that an *MDM2* promoter-specific transcription factor can be up-regulated. Such a factor would lead to direct inactivation of p53.

The *MDM2* promoter is also a direct target of p53, which is part of a negative feedback loop that down-regulates p53 (32–35). Thus, it is possible that some tumor cells that exhibit high levels of *MDM2* transcript may, in fact, actually have functional p53. Several studies, using immunohistochemical analysis, have shown that MDM2 levels are high in samples where p53 levels are elevated (36,37). Therefore, it is difficult to rule out the possibility that elevated MDM2 levels result from normal p53 signaling in these tumors. If MDM2 expression is due to normal p53 activity then, in such tumors, the p53 pathway is either intact or the pathway is inactivated at a point downstream of its immediate target genes. Alternatively, one may speculate that factors that selectively target p53 to the *MDM2* promoter, to the exclusion of other p53-responsive promoters (such as *WAF1*, *GADD45* and *BAX*), may play a role in knocking out p53 tumor suppressor activity.

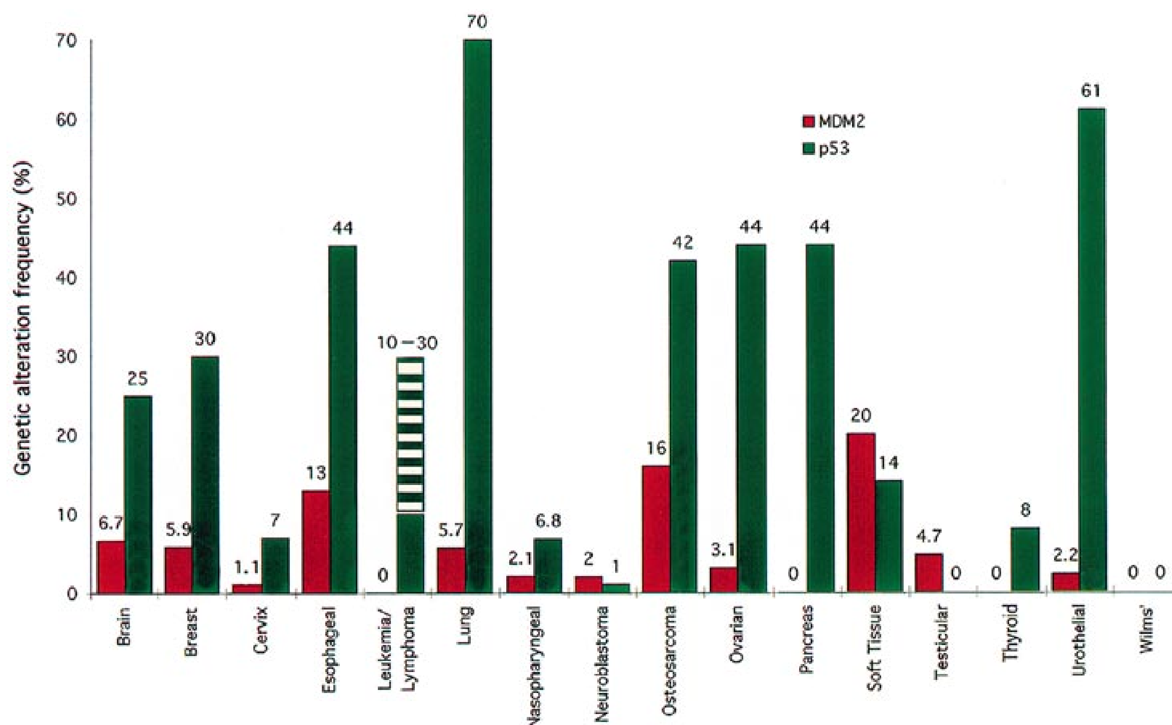

Figure 1. Comparison of *MDM2* gene amplification and *p53* mutation frequencies in human tumors. Red columns, *MDM2* amplification frequency for each tissue (data from Table 1); green columns, *p53* mutation frequency for each tissue. The number of samples for each tumor in which *p53* mutation frequencies were calculated were as follows: brain, $n = 456$ (1); breast, $n > 2400$ (13); cervix, $n = 350$ (1); esophageal, $n > 680$ (13); leukemia/lymphoma, $n > 3000$ (13); lung, $n > 1100$ (13); nasopharyngeal, $n = 117$ (96–99); neuroblastoma, $n = 212$ (1); osteosarcoma, $n = 76$ (100); ovarian, $n = 386$ (1); pancreas, $n = 170$ (1); soft tissue tumors, $n = 167$ (11,100–104); testicular, $n = 65$ (91,92); thyroid, $n = 125$ (105,106); urothelial, $n > 300$ (13); Wilms', $n = 40$ (1). Note that the stippled column indicates that the *p53* mutation frequency ranges from 10 (for leukemias) to 30% (for lymphomas).

p53-INDEPENDENT FUNCTIONS OF MDM2

MDM2 may carry out oncogenic functions independent of p53. In the initial communication describing the cloning of *MDM2* from murine cells several sizes of *MDM2* cDNAs were obtained. Subsequent sequencing indicated that *MDM2* transcripts were alternatively spliced (AS) (5). Two recent studies have shown that five human AS *MDM2* transcripts are observed in urothelial, ovarian and brain tumors (38,39). Of these five AS forms (designated a–e), only one (MDM2-e) retains p53 binding capability (Fig. 2). Interestingly, cDNAs coding for all five AS forms could independently transform NIH 3T3 cells, suggesting that these MDM2 transcripts have p53-independent transforming capability. Detectable AS forms of MDM2 transcript positively correlated with late stage forms of ovarian, urothelial and brain tumors. In brain tumors, the most prevalent AS form was MDM2-b, an AS form that retains the RING finger domain. As expected, MDM2-b was distributed randomly between tumors with mutant *p53* and tumors with wild-type *p53*. AS transcripts which express MDM2 products that are negative for p53 binding maintain the extreme N-terminal (residues 1–27) and C-terminal regions (389–491) of the protein. Interestingly, the RING finger domain near the C-terminus has been shown to bind a series of highly related RNAs (40). Whether such binding is important for p53-independent transformation is not clear.

Another unusual mechanism by which *MDM2* may contribute to tumor formation is through point mutational activation (41). It was reported that eight of 28 malignant tumor samples, which included follicular lymphomas, leukemias, hepatocellular carcinomas and an osteosarcoma, all sustained point mutations that clustered within the first putative zinc finger domain within residues 302–310 (Fig. 2). All the mutations resided within conserved domain III, a region that is extremely well conserved within *MDM2* genes cloned from five different vertebrate species (8). To date, no molecule has been identified which specifically binds this region. The mutations consist of missense, nonsense and frameshift mutations within a 27 nt stretch (nt 1217–1244), but it is unclear whether the putative protein products offer a gain of function. Seven of the eight samples carried double point mutations, indicating that this region of the gene may be hypermutable. The presence of AS forms of *MDM2* transcripts and point mutations within the *MDM2* gene could indicate that *MDM2* plays a more active role in human tumors than previously recognized.

OTHER CELLULAR MECHANISMS OF DOWN-REGULATING p53

As the study of p53 nears the end of its second decade we are gaining a clearer picture of the cellular mechanisms that regulate the *p53* pathway. The product of a recently characterized tumor suppressor gene, *ING1*, cooperates with *p53* in cell growth control (42). p53 function may be abrogated by a reduction in the level of $p33^{ING1}$, but it is too early to determine if *ING1* is inactivated in human tumors. Another protein that modulates p53 function is the product of the *INK4a* locus, $p19^{ARF}$, a protein that binds MDM2 and prevents its ability to inactivate p53 (43,44). It

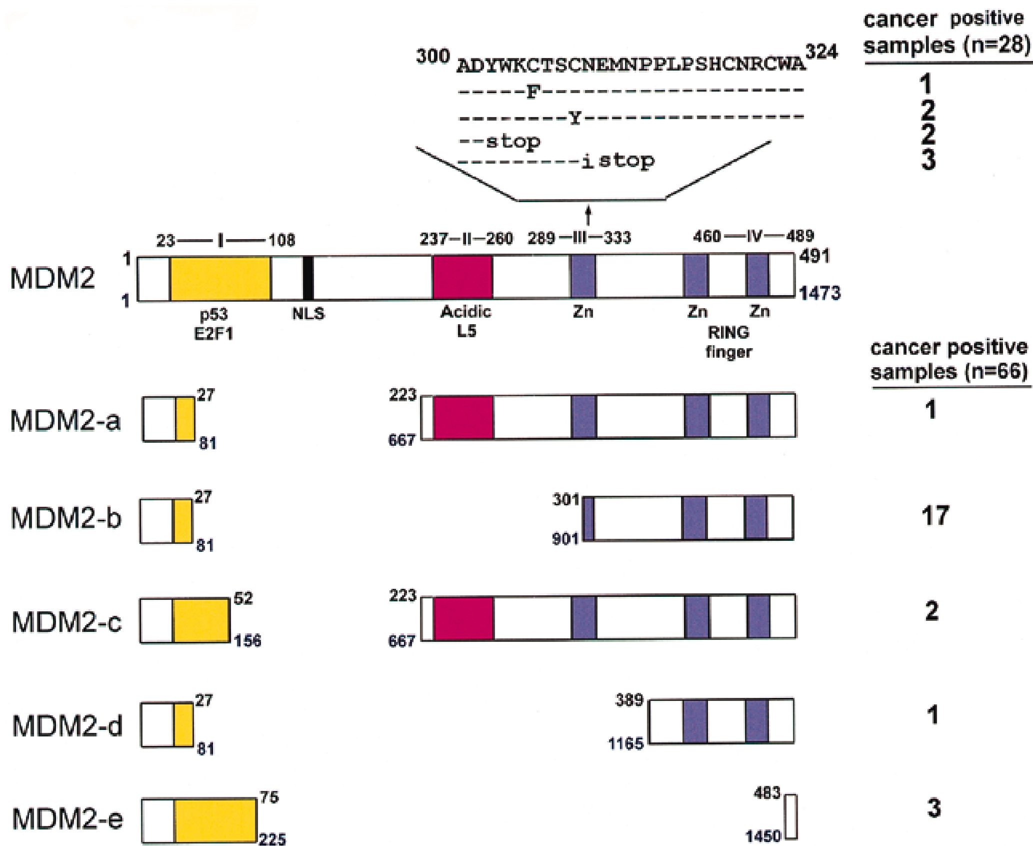

Figure 2. *MDM2* point mutations and alternatively spliced MDM2 transcripts observed in human tumors. A full-length schematic diagram of the MDM2 protein is presented. Codon numbers are listed near the top of each transcript and nucleotide numbers are listed near the bottom of each transcript. Nucleotide sequence numbers 1 and 1473 correspond to nucleotide sequence numbers 312 and 1784 in the original publication of the human *MDM2* cDNA (3). The conserved domains (I–IV) are listed above the full-length protein and color-coded regions correspond to functional domains. Yellow, p53 and E2F1 binding domain; black, putative nuclear localization sequence; red, acidic domain which binds the L5 ribosomal protein; purple, putative Zn binding motifs, the last two of which comprise the RING finger. The alternatively spliced (AS) forms of the MDM2 transcript are listed, from MDM2-a to MDM2-e (38). The distribution of the AS forms of MDM2 in brain cancers is indicated on the right. Twenty four of 66 brain cancer samples contained MDM2 AS transcripts (39). Above the full-length MDM2 protein is an expanded portion of the first Zn finger, codons 300–324. Eight of 28 short-term cultured human cancer cells contained point mutations within this small coding region (37). In this region seven point mutations were clustered. Two coded for missense protein products, two coded for a single base insertion (one caused a premature stop codon), two were silent and one coded for a premature stop codon. Dashes, identity; i, a single base insertion. The distribution of eight cancers with *MDM2* point mutations is presented on the right.

is predicted that cells with reduced $p19^{ARF}$ activity have higher MDM2 activity, leading to p53 inactivation. Interestingly, several human tumors have deletions within the *INK4a* locus that inactivate $p19^{ARF}$. However, another product of this locus, $p16^{INK4a}$ is also inactivated by such deletions. $p16^{INK4a}$ is a tumor suppressor that inactivates the cyclin D–CDK4/6 complex, which, in turn, inactivates Rb through phosphorylation (45). Thus, *INK4a* deletions may inactivate both classical tumor suppressors, p53 and Rb, at a distance. It is quite possible that other genes within the *p53* pathway are not functional in tumors (second site mutations), leaving the *p53* gene unharmed but its product deactivated.

p53 homologs may substitute for p53 function in some tissues. Three p53 homologs have recently been characterized (46–48). If these homologs parallel the p53 pathway then their inhibition would be subject to selection pressure during neoplastic transformation. The homologs may respond to cell stressors that do not activate p53, although it is difficult to find stressors that do not activate p53.

THE INTERNET DATABASE

As this is the first database that compiles the frequency of *MDM2* gene amplifications in human tumors, it was important to make it available to scientists around the globe via the World Wide Web. The database is accessible on the internet at http://www.infosci.coh.org/mdm2 . The database resides on a Microsoft SQL Server and uses Microsoft's Internet Information Server and Active Server Pages to display the data. The Web site includes an option to download the database in Access 97, Excel 97 or comma-delimited ASCII formats. Data in the *MDM2* gene amplification database are exclusively from peer-reviewed published sources and include the tumor type and subtype (when applicable), the frequency of DNA amplification and the number of samples tested. There is also an option to view a chart listing the *MDM2* amplification frequencies by tumor type and a chart comparing these frequencies with the published frequencies of *p53* mutations. This database will be updated semi-annually. Individuals who have

published studies on *MDM2* gene mutations in human tumors which are not currently included in the database may contribute to the database by sending an Email to jmomand@coh.org with their published paper reference.

THE FUTURE

Two important developments will undoubtedly occur. First, there will be a search for *MDM2* homologs that inactivate the *p53* homologs. *MDMx*, a *MDM2* homolog, may be a good candidate (49). Second, *MDM2* appears to be a likely target for cancer therapy. MDM2 can inhibit p53 activity by increasing the proteolytic susceptibility of p53 (50,51). Any tumor that has wild-type *p53*, regardless of whether *MDM2* is overexpressed, may become susceptible to p53-mediated cell cycle arrest or apoptosis if anti-*MDM2* therapy is successful. Several strategies are currently being employed, some of which are predicated on careful mapping of p53–MDM2 interaction domains and structure analysis (52,53). One strategy is to use a MDM2-targeted miniprotein (54). The miniprotein binds a cleft within MDM2 that is normally reserved for p53, thus freeing p53 to elicit proper growth control. Another strategy is to use antisense oligodeoxyribonucleotides (or phosphorothioate derivatives thereof) to prevent *MDM2* expression (55,56). Cell culture studies demonstrate that p53 activity can be regained, causing cells to undergo apoptosis or cell cycle arrest. The *MDM2* amplification database may be used as a guide to target the types of tumors that could be the first candidates for anti-*MDM2* therapy in the future.

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