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PROSPECTS

Mdm-2: "Big Brother" of p53

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Loss of cell cycle control and increased genomic instability are characteristics of most tumor cells. Activation of proto-oncogenes that promote cell growth in combination with the inactivation of tumor suppressor genes that function to inhibit cell cycle progression, leads to tumor progression and ultimately to malignancy. Perhaps one of the most prominent tumor suppressor genes is p53. Analysis of more than 5,000 human tumor samples, representing 43 different tumor types, revealed that 50–60% of all cancers have a mutated p53 gene [Greenblatt et al., 1994; Hollstein et al., 1996; Beroud et al., 1996]. What has become evident over the last few years in studying the functional properties of p53 is that its tumor suppressor activity can be inhibited in the absence of mutation. As described in this review, the Mdm-2 gene product is a p53 associated protein, that in an Orwellian fashion, monitors and negatively controls wild-type p53 activities.

WILD-TYPE P53 AND LOSS OF FUNCTION DURING TUMORIGENESIS

Wild-type p53 functions as a tumor suppressor by inhibiting oncogene mediated-cellular transformation of primary fibroblasts [Finlay et al., 1989; Eliyahu et al., 1989]. Subsequent studies revealed that p53 may function by arresting cell growth in the G1 phase of the cell cycle [Mercer et al., 1990; Michalovitz et al., 1990; Martinez et al., 1991]. A monumental effort has gone into defining the mechanisms by which wild-type p53 induces cell cycle arrest. It is now clear that wild-type p53 protein binds DNA in a sequence specific manner and activates transcription of target genes that contain p53 binding sites [for review see Zambetti and Levine, 1993]. Relevant target genes for p53 transactivation include the universal cyclin kinase inhibitor *WAF1* [El-Deiry et al., 1993; Harper et al., 1993] and *GADD45*, which is induced during DNA damage [Kastan et al., 1992]. Overexpression of either of these genes blocks cell growth in G1 [El-Deiry et al., 1993; Harper et al., 1993; Smith et al., 1994; Zhan et al., 1994].

The half-life of wild-type p53 protein is usually very short in normal cells and consequently the steady state levels of the protein are quite low. In a simplified model of p53 function, DNA damage or cellular stress results in a 3–5-fold post-transcriptional increase in the levels of p53 protein [Maltzman and Czyzyk, 1984; Kastan et al., 1991]. The elevated level of p53 induces the expression of *WAF1* and *GADD45*, which in turn initiate G1 cell cycle arrest [Kastan et al., 1992; El-Deiry et al., 1994]. When the p53 gene is inactivated, these functions are lost and the ability of the cell to properly respond to DNA damage is impaired. The inability to monitor DNA damage provides a basis for the accumulation of additional mutations that promote the tumor phenotype.

Wild-type p53 tumor suppressor function may be negatively regulated by any perturbation in the p53 signalling pathway including upstream signals and downstream effectors. Several mechanisms that inhibit p53 function have been identified and are currently under study. Inhibition of p53 tumor suppressor function can occur by: (1) mutation of the p53 gene; (2) altered subcellular localization of p53 protein; (3) specific DNA tumor virus oncogenes; and (4) the *mdm*-2 cellular proto-oncogene. The most common inactivation mechanism occurs by muta-

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tion. Analysis of the p53 gene from human tumors reveals that approximately 50% of the samples encode mutant p53 and that 85% of these mutant alleles consist of point mutations within the DNA binding core domain [Greenblatt et al., 1994]. Disruption of DNA binding prevents p53 from functioning as a transactivator and consequently inhibits its tumor suppressor activity. Loss of p53 gene expression by deletion in both alleles has also been observed in human tumors [Hollstein et al., 1996; Beroud et al., 1996]. Consistent with these observations, mice with a targeted disruption of both p53 alleles undergo normal development but form multiple tumors at approximately 3 months of age [Donehower et al., 1992; Jacks et al., 1994]. Deletion of the p53 gene resulting in an increase in tumorigenicity thus fulfills the formal definition of a tumor suppressor.

p53 protein may also be functionally inactivated by cytoplasmic sequestration [Moll et al., 1992, 1996]. Wild-type p53 must be localized in the nucleus to transactivate growth inhibitory genes to promote tumor suppression. Greater than 95% of primary human neuroblastomas overexpress wild-type p53 that is located in the cytoplasm [Moll et al., 1995]. Although the molecular mechanism for cytoplasmic localization of p53 is not yet known, functional studies have demonstrated that these cells are impaired in the induction of p53 target genes and cell cycle arrest in response to low to moderate levels of DNA damage [Moll et al., 1996]. These results suggest that this subcellular localization defect may play a role in tumor progression.

Wild-type p53 tumor suppression function can also be inhibited by several DNA tumor viruses, which encode oncoproteins that inactivate wild-type p53 protein [Levine, 1990]. For example, the E6 gene product of human papilloma virus types 16 and 18, which are associated with cervical carcinoma, specifically binds wild-type p53 and targets p53 for degradation through a ubiquitin dependent pathway [Scheffner et al., 1990; Huibregtse, et al., 1991]. Elimination of p53 protein by papilloma virus is consistent with a loss of tumor suppression.

Lastly, epidemiology studies have identified subpopulations of human tumors that maintain normal levels of wild-type p53 protein which are unaffected by mutation, altered subcellular localization, or viral oncogene products. We now know that some of these tumors may have developed, due in part to the functional inactivation of wild-type p53 by the overexpression of a cellular gene, *mdm*-2.

MDM-2 IS AN ONCOPROTEIN THAT ASSOCIATES WITH P53

The *mdm*-2 (murine double minute) gene was originally identified in a spontaneously arising tumorigenic murine Balb/c 3T3 fibroblast cell line (3T3DM) by Donna George and co-workers [Cahilly-Snyder et al., 1987]. In these cells, *mdm-2* is localized on double minute chromosomes and amplified approximately 50 fold. The *mdm-2* gene is naturally located on chromosome 12q13-14 in humans [Oliner et al., 1992] and chromosome 10 C1-C3 in mice [Cahilly-Snyder et al., 1987]. The full length human Mdm-2 protein is 491 amino acids (mouse 489 amino acids) and contains two zinc ring fingers, an acidic domain, and a putative nuclear localization signal [Fakharzadeh et al., 1991; Oliner et al., 1992; Boddy et al., 1994] (Fig. 1). The regulation of *mdm-2* gene expression is quite complex and multiple mRNA splice forms are expressed that give rise to inherently different Mdm-2 proteins [Fakharzadeh et al., 1991; Olson et al., 1993; Haines et al., 1994]. Although the biochemical function of these different mdm-2 splice forms have not yet been determined, their existence suggests that Mdm-2 may perform multiple functions. Most importantly, however, is that the *mdm-2* gene when stably transfected and amplified in normal NIH3T3 or Rat-2 cell lines, converts these cells into a tumorigenic cell line in nude mice [Fakharzadeh et al., 1991].

These characteristics suggest that *mdm-2* is a cellular proto-oncogene. What is the molecular mechanism(s) by which Mdm-2 promotes the tumorigenicity of the cell? The answer to this question became clearer when Mdm-2 was discovered to bind a temperature-sensitive p53 protein [Momand et al., 1992] in a transformed rat embryo fibroblast cell line (A1 cells) [Finlay et al., 1988; Michalovitz et al., 1990; Martinez et al., 1991]. At the non-permissive temperature (39.5°C) the p53 protein is in a mutant conformation in the cytoplasm and the cells grow at an exponential rate. In contrast, the p53 protein is in the wild-type conformation when the cells are incubated at the permissive temperature (32.5°C). Under these conditions, p53 accumulates in the nucleus and the cells arrest in the G1 phase of the cell cycle. Screening for p53 associated proteins by immunopre-

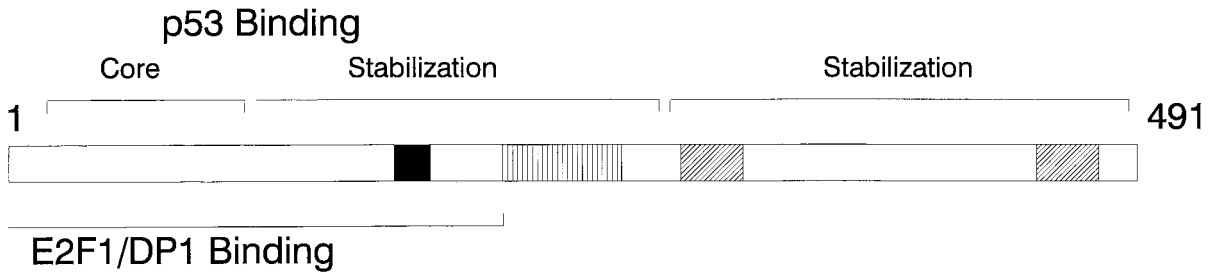

Fig. 1. Schematic diagram of the primary structure of human Mdm-2. The full length human Mdm-2 protein is 491 amino acids and contains a putative nuclear localization signal (aa 181–185; black box), two zinc fingers (aa305–322 and aa461–478; diagonally lined boxes), and an acidic domain (aa223–274; vertical lined box) [Fakharzadeh et al., 1991; Oliner et al., 1992]. Murine Mdm-2 is 389 amino acids [Fakharzadeh et al., 1991] and is 80% homologous to human mdm-2 [Oliner et al., 1992]. The p53 protein-binding core domain of human Mdm-2 (aa19–102) is essential but not sufficient for stable protein-protein interactions [Chen et al., 1993]. Sequences outside this domain (aa102–294 and/or aa294–491) are additionally required for stable interactions [Chen et al., 1993]. The E2F1/DP1 protein-binding domain of Mdm-2 spans aa1-220.

cipitation analysis of radiolabeled cells grown at low temperature identified a 90 kD protein that co-immunoprecipitated with p53 [Momand et al., 1992; Barak and Oren, 1992]. Subsequent microsequence analysis of this associated protein led to its identification as Mdm-2 [Momand et al., 1992].

MDM-2 INHIBITS WILD-TYPE P53 TUMOR SUPPRESSION FUNCTION

In light of the oncogenic properties of Mdm-2 and its association with wild-type p53, it was postulated that Mdm-2 may promote the tumor phenotype by inactivating the tumor suppressor function of p53. Since p53 transactivation correlated with its growth suppression property, the effects of *mdm-2* gene expression on wild-type p53 transactivation was examined [Momand et al., 1992]. Transfection of cells with a cosmid that expresses Mdm-2 completely abolished wild-type p53 transactivation of a reporter gene containing a p53 response element. This result represented the first demonstration that Mdm-2 acts in a trans-dominant negative manner to inhibit wild-type p53 transactivation function [Momand et al., 1992]. The mechanism by which Mdm-2 inhibits wild-type p53 transactivation function appears to occur by concealing the acidic domain of p53 [Oliner et al., 1993; Chen et al., 1993; Picksley et al., 1994]. Other evidence suggests that Mdm-2 may also prevent p53 from binding DNA [Zauberman et al., 1993].

Mdm-2 negatively regulates wild-type p53 mediated transcription by directly binding the p53 protein. Mapping studies have localized this site of interaction to the N-terminus of both Mdm-2 and p53. The Mdm-2 binding site on human p53 spans amino acids 18–23 (TFSDLW) based on in vitro translation/immunoprecipitation analysis [Chen et al., 1993], structure and function analysis [Oliner et al., 1993; Lin et al., 1994; Leng et al., 1995] and fine mapping studies using short synthetic peptides [Picksley et al., 1994]. Interestingly, this site on p53 interacts with several other important factors which play a role in mediating or regulating wild-type p53 function, such as the TATA binding protein (TBP) [Seto et al., 1992; Liu et al., 1993; Truant et al., 1993; Horikoshi et al., 1995], transcription associated factors $hTAF_{II}32$ and $hTAF_{II}70$ [Thut et al., 1995; Lu and Levine, 1995], and the adenovirus E1B 55kD oncoprotein [Lin et al., 1994]. Partial overlap of the binding sites for Mdm-2, TBP, and the TAFs on the acidic domain of p53 provides a biochemical explanation for how Mdm-2 may inhibit p53-mediated transactivation.

The Mdm-2 gene product: (1) physically associates with p53; (2) inhibits wild-type p53 transactivation function; and (3) is transforming when overexpressed in murine fibroblasts. Based on these findings it was proposed that Mdm-2 may be oncogenic, in part, by abolishing p53 tumor suppressor activity. This hypothesis was tested and validated by immortalization

and transformation assays. Transfection of primary rat embryo fibroblasts with cooperating oncogenes, such as E1A and activated H-*ras*, results in the selection of fully transformed cell lines. When wild-type p53 expression constructs are included with these cooperating oncogenes, the number of transformed foci is dramatically reduced [Finlay et al., 1989; Eliyahu et al., 1989; Hinds et al., 1990]. The few foci that develop either do not express the p53 protein or they express a mutated form of the exogenous p53 gene. The *mdm-2* gene can substitute for E1A as a cooperating oncogene in primary fibroblast transformation assays [Finlay, 1993]. However, in sharp contrast to transformation with H-*ras* and E1A, which does not bind or inactivate p53, the foci that result from transfection with H-*ras*, *mdm-2*, and p53 maintain expression of wild-type p53 protein in more than half of the resulting cell lines [Finlay, 1993]. Furthermore, transfection of primary rat embryo fibroblasts with *mdm-2* alone efficiently immortalized these cells [Finlay, 1993], which is consistent with previous observations that wild-type p53 is functionally inactivated during immortalization [Harvey and Levine, 1991]. These results demonstrate that Mdm-2 abrogates wild-type p53 tumor suppression activity.

Abrogation of wild-type p53 tumor suppression function by Mdm-2 may be a consequence of blocking wild-type p53 induced apoptosis (cell suicide) [Yonish-Rouach et al., 1991]. Wild-type p53 has been shown to elicit an apoptotic response during DNA damage or hypoxia in certain cell types [Lowe et al., 1993; Clarke et al., 1993; Graeber et al., 1995]. Induction of apoptosis may be critical to p53 mediated-tumor suppression and inhibition of this function in tumors could provide a distinct growth advantage. Consistent with this concept, Kondo et al. [1995] demonstrated that overexpression of *mdm-2* in a glioblastoma cell line inhibited p53-mediated apoptosis in response to DNA damage induced by cisplatin. In support of these findings, inhibition of p53-mediated apoptosis by Mdm-2 has also been observed in other cell systems [Chen et al., 1996; Haupt et al., 1996]. These results suggest that tumors that express high levels of Mdm-2 may be less susceptible to apoptosis during chemotherapy and therefore, more prone to drug resistance.

MDM-2 EXPRESSION IN TUMORS

Cellular transformation of rat embryo fibroblasts and inhibition of wild-type p53 tumor suppression activity by Mdm-2 predicts that in some human cancers *mdm-2* may function as an oncogene to promote the tumorigenicity of the cell. In addition, the model predicts that wild-type p53 may be tolerated in these tumors due to inactivation by the overexpression of *mdm-2*. Consistent with this possibility, the chromosomal position of *mdm-2* (12q13–14) is frequently altered in human sarcomas [Oliner et al., 1992]. Subsequent analysis of human soft tissue tumors (including liposarcomas, malignant fibrous histiocytomas, and osteosarcomas) demonstrated that the *mdm-2* gene was amplified in approximately 20% of the samples (Table I). In primary sarcomas and cell lines that were characterized in more detail, amplification of the *mdm-2* gene correlated with overexpression of the Mdm-2 protein, while maintaining the wild-type p53 genotype [Oliner et al., 1992]. Furthermore, in a limited sample study, *mdm-2* gene amplification was more frequently observed in metastatic or recurrent human osteosarcoma than in primary osteosarcomas [Ladanyi et al., 1993], implicating a role for Mdm-2 in late stage tumor progression. Amplification of *mdm-2* has also been detected in other tumor types, including human malignant gliomas, breast cancers and non-small cell lung carcinomas (Table I). *Mdm-2* amplification appears to be more common in tumor cells of non-epithelial origin, especially those derived from the mesenchyme. Interestingly, amplification of *mdm-2* may be associated with higher grade tumors due to co-amplification with other proto-oncogenes, such as *CDK4* and *GLI* [Khatib et al., 1993].

Mdm-2 overexpression may also be achieved independently of gene amplification. A survey of human leukemia samples (including a variety of cell lines and primary leukemias) demonstrated that the *mdm-2* gene may be maintained at a normal copy number while the mdm-2 mRNA is dramatically upregulated [Bueso-Ramos et al., 1993; Watanabe et al., 1994]. In addition, there is an example of a murine plasmacytoma cell line that harbors a translocation of *mdm-2* to the immunoglobulin C kappa gene, which gives rise to elevated levels of mdm-2 mRNA [Berberich and Cole, 1994]. Other cell lines maintain normal, low levels of mdm-2 mRNA but express high levels of Mdm-2 protein, apparently through enhanced translation efficiency [Landers et al., 1994]. Although the molecular mechanisms of

TABLE I. Mdm-2 Amplification in Human Tumors

Cancer type	MDM2 amplification percent (n)	References[a]
Breast	4.1 (194)	12, 13, 14
Brain tumors	8.6 (140)	1, 2, 18
Astrocytoma	11 (27)	1
Glioblastoma	7.6 (105)	1, 2
Medulloblastoma	0 (8)	18
Esophageal	13 (96)	3, 28
Leukemia/lymphoma	0 (304)	15, 16, 17
Lung cancer (NSCLC)	5.7 (53)	27
Neuroblastoma	2.0 (51)	6, 23, 24
Osteosarcoma	9.7 (124)	5, 8, 9, 26
Ovarian	0 (32)	19
Soft tissue tumors	20 (375)	4–11
Leiomyosarcoma	0 (17)	5, 7
Lipoma (benign)	30 (64)	7, 8
Myxoid lipoma	0 (16)	7
Liposarcoma	35 (60)	5, 8–10
Malignant fibrous histiocytoma	19 (111)	5, 7–9
Malignant schwannoma	19 (16)	5
Testicular germ cell tumors	12 (25)	25
Urothelial	2.2 (137)	20, 21
Uterine cervix	0 (35)	22
Wilm's tumor	0 (40)	6

[a]References: 1. Reifenberger et al., 1993; 2. He et al., 1994; 3. Esteve et al., 1993; 4. Ladanyi et al., 1995; 5. Florenes et al., 1994; 6. Waber et al., 1993; 7. Nilbert et al., 1994; 8. Nakayama et al., 1995; 9. Oliner et al., 1992; 10. Pedeutour et al., 1994; 11. Cordon-Cardo et al., 1994; 12. McCann et al., 1995; 13. Marchetti et al., 1995; 14. Quesnel et al., 1994a; 15. Quesnel et al., 1994b; 16. Ridge et al., 1994; 17. Maestro et al., 1995; 18. Adesina et al., 1994; 19. Foulkes et al., 1995; 20. Habuchi et al., 1994; 21. Lianes et al., 1994; 22. Kessis et al., 1993; 23. Moll et al., 1995; 24. Corvi et al., 1995; 25. Riou et al., 1995. 26. Ladanyi et al., 1993; 27. Marchetti et al., 1995b; 28. Shibagaki et al., 1995.

deregulation of human *mdm-2* gene expression remain to be determined, these results clearly implicate *mdm-2* as a proto-oncogene that promotes the tumorigenicity of the cell, at least in part, by binding to p53 and inactivating its tumor suppression function.

FUNCTIONAL INTER-RELATIONSHIP BETWEEN MDM-2 AND P53

The strategy for purification of the Mdm-2 protein [Momand et al., 1992] exploited the observation that the A1 cell line, which expresses a temperature-sensitive mutant p53 [Martinez et al., 1991], yielded much higher levels of Mdm-2 in complex with p53 when the p53 protein was in the wild-type conformation. These results suggested that Mdm-2 may specifically recognize the wild-type p53 conformation or interact with p53 within the nucleus (wild-type p53 is nuclear whereas mutant p53 is cytoplasmic in these cells). Alternatively, *mdm-2* gene expression may be induced by wild-type p53 so that the elevated levels of the Mdm-2 protein would be available for complex formation. Indeed, Northern and Western blot analysis demonstrated that *mdm-2* gene expression is induced in response to wild-type p53 [Wu et al., 1993; Barak et al., 1993; Otto and Deppert, 1993]. The *mdm-2* gene contains two imperfect p53 DNA binding sites located in intron 1 and these sites confer wild-type p53 responsiveness to heterologous reporters. These results, taken together with earlier studies demonstrating that Mdm-2 inhibits wild-type p53 transactivation, establish an important autoregulatory feedback loop for wild-type p53 function. DNA damage induces wild-type p53 protein, which serves to transactivate the appropriate target genes such as *WAF1* [El-Deiry et al., 1993; Harper et al., 1993], *GADD45* [Kastan et al., 1992], and *mdm-2* [Perry et al., 1993; Price and Park, 1994]. The induced Mdm-2 protein enters into complex with p53 resulting in the down regulation of the p53-mediated growth suppressive response. However, the model is perhaps not so simple. DNA damage of cells expressing wild-type p53, using high doses of ultraviolet irradiation, results in a rapid accumulation of p53 protein followed by a delayed induction of *mdm-2* gene expression [Perry et al., 1993]. In agreement with the autoregulatory model, the induction of Mdm-2 in these cells is dependent on wild-type p53 function. The delay in *mdm-2* gene expression, despite the rapid induction of wild-type p53, suggests that there exists another level of regulating wild-type p53 transactivation function and this molecular mechanism remains to be determined.

Induction of Mdm-2, by virtue of its ability to complex with p53, down-regulates wild-type p53 transactivation function, thus creating a balance between the tumor suppressor and the oncogene. The importance of this balance has been recently demonstrated by correlating the level of free Mdm-2, free p53, and Mdm-2/p53 complex to cell growth and tumorigenicity [Momand and Zambetti, 1996]. For example, a cell line that overexpresses Mdm-2, contains no de-

tectable wild-type p53 protein that is not bound to Mdm-2, actively proliferates, and is tumorigenic. In contrast, a cell line that artificially overexpresses wild-type p53 overrides Mdm-2 inhibition by accumulating free p53 protein and arrests in the G1 phase of the cell cycle [Momand and Zambetti, 1996]. In normal proliferating cells, however, low levels of p53 and Mdm-2 are present but no complex is formed. Furthermore, cells that overexpress Mdm-2 are incapable of arresting in G1 in response to wild-type p53 [Chen et al., 1996]. These results illustrate the functional relationship between wild-type p53 and Mdm-2, specifically the absolute level of free p53, and demonstrate that perturbations of this balance impact the growth state of the cell.

Autoregulation of wild-type p53 function by Mdm-2 appears to have developed as a safeguard against uncontrolled p53 activity. Constitutive expression of wild-type p53 function may result in permanent cell growth arrest or apoptosis, which would be detrimental to normal cellular processes and development. Earlier studies demonstrated that the loss of wild-type p53 function in p53-knockout mice promotes tumor progression [Donehower et al., 1992; Jacks et al., 1994]. What is the consequence of loss of *mdm-2* gene expression in mice that express wild-type p53? This question has been recently addressed in two elegant studies demonstrating that the *mdm-2*-null genotype leads to embryonic lethality [Montes de Oca Luna et al., 1995; Jones et al., 1995]. *mdm-2* +/− heterozygote mice are viable, develop normally, and are fertile. Interbreeding of *mdm-2* +/− heterozygotes failed to result in offspring with the *mdm-2* −/− genotype, suggesting that the absence of Mdm-2 during fetal development is lethal. Histological analysis of day 5.5 embryos from *mdm-2* +/− intercrosses revealed that approximately one-quarter of the embryos (presumably *mdm-2* −/−) had very few cells, if any, within the maternal decidua. An intriguing question that arises from these studies is whether the absence of Mdm-2 induces loss of viability due to deregulated wild-type p53 function or to p53 independent pathways. Crossing *mdm-2* +/− mice with p53 +/− or p53 −/− mice resulted in progeny that were homozygous for both *mdm-2* and p53 null alleles and these mice developed normally, not unlike the p53 null mice. In addition, no mice were recovered that were null for *mdm-2* in a wild-type or heterozygous p53 background. These results demonstrate in a most convincing manner that a primary developmental role for Mdm-2 is to negatively regulate wild-type p53 function.

ADDITIONAL FUNCTIONS OF MDM-2

The results from the *mdm-2* knockout mouse model clearly demonstrate the functional interaction of Mdm-2 and p53. These results, however, do not exclude the possibility that Mdm-2 may have additional functions that are not manifested due to the early embryonic lethality of the animals in the wild-type p53 background. Recent data suggest that Mdm-2 may have biochemical properties other than inactivating wild-type p53, such as binding to the retinoblastoma tumor suppressor protein (RB) [Xiao et al., 1995], the RB associated E2F1 protein and the E2F1 associated DP1 protein [Martin et al., 1995]. Complex formation between Mdm-2 and RB has been demonstrated both in vivo and in vitro and the functional consequence of this interaction is the inhibition of RB growth repression function [Xiao et al., 1995]. Deletion analysis demonstrated that the C-terminus of RB (aa792–928) mediates Mdm-2 binding [Xiao et al., 1995]. Based on in vitro translation/co-immunoprecipitation assays, the N-terminus of Mdm-2 (aa1-220) binds E2F1 (aa359–407) and DP1 (for optimal binding aa59–331; sufficient for binding aa226–375) [Martin et al., 1995]. In addition, the physical interaction of Mdm-2 with DP1 has been observed in vivo. In agreement with the protein binding data, Mdm-2 functionally interacts with RB, E2F1, and DP1 to stimulate E2F1 mediated transactivation of a responsive reporter in transient transfection assays [Xiao et al., 1995; Martin et al., 1995]. Mdm-2 can also overcome G1 growth arrest mediated by p107, a member of the RB tumor suppressor gene family. Dubs-Poterszman et al. [1995] demonstrated that Mdm-2 overexpression in SaOs-2 cells, a human osteogenic sarcoma that does not express p53, resulted in an increase in the number and size of soft agar colonies. These results suggest that in addition to Mdm-2 inhibition of wild-type p53 growth suppression activity, the Mdm-2 protein may also promote cell cycle progression by enhancing the growth accelerating effects of the E2F/DP1 complex.

FUTURE DIRECTIONS AND CONSIDERATIONS

In less than 4 years, a series of landmark papers have been published that clearly estab-

lish *mdm-2* as an oncogene that promotes the tumor phenotype, at least in part, by antagonizing wild-type p53 tumor suppressor function. Indeed, the analogy of Mdm-2 to George Orwell's "Big Brother" is based on the function of Mdm-2 in the surveillance and negative regulation of p53. Additional studies are needed to define the role of Mdm-2 in promoting tumorigenesis along p53 dependent and independent pathways and to determine whether wild-type p53 may negatively regulate putative Mdm-2 functions. The answers to these problems, as well as others, should be provided by the power of murine genetics. For example, what is the spectrum of tumor formation in homozygous *mdm-2* −/− and p53 −/− double knockout mice? If p53 independent pathways are involved in *mdm-2* functioning as an oncogene then one would predict that the incidence of tumors would be lower in these mice. Conversely, what is the phenotype of p53 null mice that overexpress an *mdm-2* transgene? Are these mice more prone to tumor formation?

Most importantly, what do we do with this information. Approximately 20% of all soft tissue tumors overexpress Mdm-2 and preliminary evidence indicates that there is a good correlation between Mdm-2 overexpression and poor prognosis [Cordon-Cardo et al., 1994]. Disruption of the interaction of Mdm-2 with wild-type p53 and perhaps RB, E2F1, and DP1 should be an important goal for cancer therapy.

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