Molecular Pathways: Targeting Mdm2 and Mdm4 in Cancer Therapy

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Abstract

The p53 tumor suppressor is activated in response to cellular stresses to induce cell-cycle arrest, cellular senescence, and apoptosis. The p53 gene is inactivated by mutations in more than 50% of human tumors. In addition, tumor cells dampen p53 activities via overexpression of p53-negative regulators, in particular 2 structurally related proteins, Mdm2 and Mdm4. And yet, Mdm2 and Mdm4 possess p53-independent activities, which also contribute to tumor formation and progression. Given that Mdm2 and Mdm4 inhibit p53 activities to promote tumor development, small molecules and peptides were developed to abrogate the inhibition of p53 by Mdm proteins. Antitumor activities of these molecules have already been confirmed in preclinical studies and early-phase clinical trials. These research endeavors and clinical advances constitute the main focus of this review. Clin Cancer Res; 19(1); 1–8. ©2012 AACR.

Background

p53, the most frequently inactivated gene in human cancers, is mutated in more than half of human tumors. In response to various extra- and intracellular stresses including but not limited to oncogene activation, DNA damage, and hypoxia, p53 functions as a transcriptional factor and transactivates a set of genes engaged in multiple cellular processes such as cell-cycle arrest, cellular senescence, energy metabolism, and apoptosis. p53 signaling is also alternatively inactivated by high levels of p53 inhibitors. Suppression of p53 activities is largely attributed to 2 major negative regulators of p53, Mdm2 and Mdm4 (reviewed in ref. 1). Different mechanisms increase the levels of Mdm2 and/or Mdm4 in a large number of tumors that carry wild-type p53 alleles. These observations suggest that upregulation of Mdm2 and/or Mdm4 serves as an alternate means of inactivating the p53 pathway, further highlighting that silencing p53 signaling is a more common event in tumorigenesis than previously thought.

In this review, we will discuss the regulation of p53 by the Mdm proteins, p53-independent oncogenic functions of Mdm2 and Mdm4, as well as potential pharmaceuticals targeting Mdm2 and Mdm4 in cancer treatment.

Regulation of p53 Functions by Mdm2 and Mdm4

The murine double minute 2 (Mdm2) was first identified as one of the genes that were amplified on double minute chromosomes in transformed mouse NIH-3T3 fibroblasts (2). Early studies showed that Mdm2 could bind the p53 transactivation domain and repress its transcriptional activity (3, 4). In addition, Mdm2 functions as an E3 ubiquitin ligase, mediates ubiquitination of p53, and targets it for degradation (reviewed in ref. 1). By exploiting the beauty of genetic models, 2 research groups independently showed that biallelic deletion of p53 rescued the embryonic lethal phenotypes in Mdm2-deficient embryos (5, 6). Deletion of Mdm2 in specific types of cells such as neuronal progenitors and cardiomyocytes also leads to p53-dependent embryonic lethal phenotypes (7–9). These in vitro and in vivo data together suggest an essential function of Mdm2 as a negative regulator of p53 in numerous cell types.

Mdm4 was originally discovered as a p53-interacting protein through screening of a mouse embryo cDNA expression library (10). The Mdm4 and Mdm2 proteins are very similar at the primary structural level (10, 11). The p53-binding domains at the N-termini of Mdm4 and Mdm2 show very high structural and functional similarities (10). The p53-binding domain of Mdm4, like that of Mdm2, interacts with the transactivation domain of p53 to repress its transcriptional activity (10). Another prominent conserved domain between Mdm4 and Mdm2 is the RING-finger domain at the C-termini of both proteins (10, 11). The RING domain of Mdm4 is responsible for its E3 ubiquitin ligase function (12); however, the Mdm4 RING domain lacks E3 ligase activity (reviewed in ref. 1). Mice with Mdm4 RING domain alterations recently revealed that interaction of Mdm2 with Mdm4 through this RING domain is required for modulating p53 activities in embryonic stages but dispensable for Mdm2 and p53 stabilization in the adult mouse (13, 14). Loss of Mdm4 in mice also results in embryonic lethality, which is completely rescued by concomitant p53 loss (15–17). These data indicate that Mdm4 negatively regulates p53...
activity in vivo. Deletion of Mdm4 in mouse erythroid progenitors, embryonic neuronal progenitors, cardiomyocytes, and intestinal epithelia leads to distinct pathologic phenotypes, compared with that of Mdm2 (18–22). Deletion of both Mdm2 and Mdm4 in mouse embryonic neuronal progenitors results in a more severe phenotype compared with deletion of either gene alone (8). All Mdm loss-of-function phenotypes are rescued by deletion of p53 (7, 8, 18–20). Together, these data suggest that Mdm2 and Mdm4 function in a nonoverlapping manner to suppress p53 activities in vivo. Intriguingly, overexpression of Mdm2 also rescues the Mdm4-/- embryonic lethality (23), implying that high levels of Mdm2 can compensate for loss of Mdm4, possibly through inhibition of p53 activities.

p53-Independent Functions of Mdm2 and Mdm4

Although the major function of Mdm2 is to suppress p53 activities, emerging evidence has identified p53-independent roles of Mdm2 in tumor formation and progression. Early studies showed that p53-/- mice carrying an Mdm2 transgene developed a higher percentage of sarcomas compared with p53-/- mice (24), supporting the p53-independent functions of Mdm2 in tumorigenesis. p53-independent activities of Mdm2 include regulation of genomic instability, apoptosis, and metastasis.

Elevated genomic instability is a hallmark of cancers (reviewed in ref. 25). Ectopic expression of Mdm2 in murine mammary epithelial cells and murine embryonic fibroblasts (MEF) increases ploidy and chromosome/chromatid breaks regardless of p53 status (reviewed in ref. 26), suggesting that Mdm2 promotes genomic instability independently of p53. Mdm2 induces genomic instability likely through inhibiting DNA damage repair and suppressing cell-cycle arrest. The repair of DNA double-strand breaks (DSB) caused by ionizing radiation (IR) requires the Mre11 complex, composed of Mre11, Rad50, and Nbs1 (27). Mdm2 directly binds and colocalizes with Nbs1 at DNA damage sites after IR treatment, and in turn inhibits DNA DSB repair (ref. 28; Fig. 1A). The p53-independent functions of Mdm2 in suppressing cell-cycle arrest involve the direct interaction with Retinoblastoma protein (Rb) and E2F transcription factors (Fig. 1A). The Rb protein interacts with several E2Fs, suppresses E2F-mediated transcription of cell-cycle essential genes, and therefore causes cell-cycle arrest especially at the G1–S transition (reviewed in ref. 29). Loss of Rb activity leads to genomic instability and tumorigenesis due to defective cell proliferation and chromosome missegregation (30). Mdm2 compromises Rb-mediated G1 arrest through multiple mechanisms: (i) Mdm2 directly binds Rb protein, suppresses the interaction between Rb and E2F1, and induces activation of E2F1; and (ii) Mdm2 targets Rb for ubiquitin-dependent and -independent degradation (reviewed in ref. 26; Fig. 1A). In addition, Mdm2 also directly binds E2F1 and activates its transcriptional activities (ref. 31; Fig. 1A). Moreover, Mdm2

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**Figure 1.** Schematic representation of p53-independent functions of Mdm2 and Mdm4. A, Mdm2 interacts with multiple factors to affect genomic instability, apoptosis, and metastasis. hnRNP, hnRNP K protein. B, Mdm4 interacts with p21 and E2F1 to regulate cell-cycle arrest and apoptosis.

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targets other cell-cycle inhibitors, such as p21 and hnRNP K, for proteasomal degradation, and therefore promotes cell proliferation (reviewed in refs. 22, 26; Fig. 1A). Notably, p21 is involved in maintaining genomic stability (32), and loss of p21 induces aneuploidy, chromosomal aberrations, and accelerated tumor onset in mice (33). These data suggest that degradation of p21 by Mdm2 contributes to genomic aberrations (Fig. 1A). Thus, Mdm2 binds and alters the activities of several proteins involved in DNA repair and transition through the cell-cycle impacting ploidy and other chromosomal abnormalities.

Additional data indicate a role of Mdm2 in inhibition of apoptosis independently of p53. FOXO3a, a forkhead transcription factor, induces apoptosis and decreases tumorigenicity of breast cancer cells (34), and inverse correlation between Mdm2 and FOXO3a expression exists in human breast cancer samples, because of Mdm2-mediated degradation of FOXO3a (ref. 34; Fig. 1A), indicating that Mdm2 may counteract FOXO3a-mediated apoptosis during tumor development. Unexpectedly, Mdm2 was also reported to suppress apoptosis via a different pathway by upregulating the antiapoptotic protein XIAP through translational regulation (ref. 35; Fig. 1A). Upon DNA damage, cytoplasmic Mdm2 binds the internal ribosome entry site at the XIAP 5′-UTR and enhances the translation of XIAP mRNA, which may confer resistance to radiation-induced apoptosis in tumor cells (35). The mechanism by which Mdm2 regulates this process is unknown.

Additional p53-independent functions of Mdm2 include the ability to promote epithelial-to-mesenchymal transition (EMT) and metastasis (Fig. 1A). The EMT effector TGF-β1 induces Mdm2 transcription via activation of Smad3 in murine mammary epithelial cells (36). Histopathologic analyses of human breast cancers indicate that activation of Smad3 and overexpression of Mdm2 coexist in 65% of early-stage carcinomas and are strongly correlated to metastatic phenotypes in patients with breast cancer (36), suggesting an important role of Mdm2 overexpression in metastasis. A mechanism by which Mdm2 promotes EMT and metastasis is through E-cadherin. Mdm2 binds E-cadherin, which targets it for degradation, thus inducing EMT (ref. 37; Fig. 1A). Overexpression of Mdm2 in breast cancer cells disrupts cell–cell contacts, enhances cell motility, and promotes cell invasiveness (37). High Mdm2 levels are also strongly associated with low E-cadherin levels in human metastatic breast cancer specimens (37). Thus, a series of experiments in breast cancers indicate that high levels of Mdm2 correlate with metastasis.

Data for p53-independent functions of Mdm4 are, by far, more limited. Mdm4 was found to interact with p21 and target it for proteasomal degradation independently of ubiquitination (ref. 38; Fig. 1B). Degradation of p21 mediated by Mdm4 is independent of, but in cooperation with, Mdm2 and leads to abrogation of G1 cell-cycle arrest (38). Surprisingly, loss of Mdm4 in p53-null cells leads to multipolar spindle formation, enhanced loss of chromosomes, elevated proliferation potentials, and increased spontaneous transformation as compared with p53+/− cells (39). In addition, deletion of Mdm4 results in accelerated tumorigenesis in both p53+/− and p53−/− mice (39). As Mdm4 forms heterodimers with Mdm2 (reviewed in ref. 1), loss of Mdm4 may promote interaction of Mdm2 with other proteins such as Rb and p21 and thus enhances tumorigenesis. Moreover, Mdm4 represses E2F1 transactivation by either disrupting E2F1-DNA binding (40) or altering localization of E2F1 transcription complex (ref. 41; Fig. 1B). Overexpression of E2F1 promotes G1→S transition in cells, and is associated with tumorigenesis (42). However, upregulation of E2F1 also triggers both p53-dependent and-independent apoptosis (reviewed in ref. 43). These data together suggest that upregulation of Mdm4 that is present in many human tumors (44), in addition to suppressing p53 activity, may represent a mechanism for tumor cells to overcome apoptosis induced by elevated E2F1 levels.

### Elevated Levels of Mdm Proteins in Human Tumors

The functions of Mdm2 and Mdm4 including p53 inhibition and p53-independent activities all suggest important roles in tumorigenesis and tumor progression. High levels of Mdm2 are commonly observed in human cancers including sarcomas, gliomas, hematologic malignancies, melanomas, and carcinomas (reviewed in ref. 45). By analyzing gene amplification and overexpression status of Mdm2 in human soft tissue tumors, Patterson and colleagues (46) showed that high levels of Mdm2 detected by immunohistochemistry occurred in tumors with Mdm2 gene amplification, but were also found in tumors without Mdm2 gene amplification (46). This observation indicates that Mdm2 gene amplification indeed results in its overexpression, but other mechanisms may also confer Mdm2 upregulation. Extensive studies have indicated that upregulation of Mdm2 results from elevated transcription, increased mRNA stability, enhanced translation, and altered posttranslational modification (reviewed in refs. 22, 47). In addition, these mechanisms are associated with upregulation of Mdm2 in tumors.

High levels of Mdm4 are also found in a variety of human cancers. Mdm4 upregulation in malignancies is mostly ascribed to Mdm4 gene amplification (reviewed in ref. 44). Gene amplification and consequent upregulation of Mdm4 are reported in multiple types of human tumors including glioma, soft tissue sarcoma, head and neck squamous carcinoma, retinoblastoma, melanoma, and breast cancer (reviewed in ref. 44). To date, information about transcriptional regulation of Mdm4 remains extremely limited. Stimulation with mitogens, for example, IGF-1, leads to phosphorylation of mitogen-activated protein kinase (MAPK) ERK and thus activates transcriptional factors c-Ets-1 and Elk-1, which bind the Mdm4 promoter and induce Mdm4 expression (48). Human colon tumors that are stained positive for phosphorylated ERK are 2-fold more likely to have high levels of Mdm4 (48), implying a role of Mdm4 overexpression stimulated by the MAPK in tumor
development. Mdm4-S, the first Mdm4 transcript variant identified in human and mouse cells, produces an Mdm4 isoform containing the p53-binding domain but missing the negative regulatory sites, which is more stable and oncogenic than full-length Mdm4 (reviewed in ref. 49). Overexpression of Mdm4-S has been found in several types of cancers, and is associated with decreased survival and poor prognosis for patients with cancer (reviewed in refs. 44, 49), indicating a role of Mdm4-S in tumor development.

A number of studies have modeled Mdm2 and Mdm4 overexpression in vivo using genetically engineered mice. Transgenic mice overexpressing Mdm2/Mdm4 either globally or in specific tissues/cell types all develop spontaneous tumors (24, 50–54). A T to G single-nucleotide polymorphism (SNP309) in the human Mdm2 P2 promoter creates an additional binding site for the transcriptional activator Sp1, leads to elevated Mdm2 expression, and attenuates p53 signaling (55). An association between SNP309 and earlier tumor onset is revealed in both patients with hereditary and sporadic cancer (55). A knockin mouse with a humanized Mdm2 intron 1 harboring SNP309 was generated in our laboratory and develops multiple types of malignancies with accelerated onset in both p53 wild-type and p53 mutant backgrounds, providing causal evidence for the role SNP309 in tumorigenesis (54). Approximately 65% of human retinoblastomas undergo gene amplification of Mdm4 and 10% have extra copies of Mdm2, but no p53 mutations or defects downstream to p53 are found in the retinoblastomas caused by Rb loss (56), suggesting that p53 is inactivated in retinoblastomas via overexpression of p53 negative regulators. A very low retinoblastoma penetrance is observed in mice with loss of Rb family genes Rb and p107 in retina (56), but ectopic expression of Mdm4 in retina significantly promotes retinoblastoma development in these mice with Rb and p107 loss (56). In addition, the treatment of mice with p53-activating drugs leads to growth suppression of Mdm4-overexpressing retinoblastomas (56), suggesting that p53 remains intact in these tumors. Therefore, this retinoblastoma mouse model recapitulates human defects and emphasizes the causative role of Mdm4 overexpression in retinoblastoma formation. In a recent study, Mdm4 is overexpressed in 65% of human melanoma samples examined, and p53 mutations are rare among these melanoma cases (57). Specific overexpression of Mdm4 in the murine melanocytes enhances tumorigenesis of Nras-induced melanoma in both p53 wild-type and heterozygous backgrounds, whereas knockdown of Mdm4 in human melanoma cells leads to increased cell death but reduced cell proliferation and oncogenic ability (57). However, the p53 status in these mouse melanomas has not been examined. Nevertheless, this melanoma mouse model elucidates the tumorigenic roles of Mdm4 overexpression in melanoma formation in vivo.

Clinical–Translational Advances

Targeting Mdm proteins in cancer therapy

Clearly, Mdm proteins are present at high levels and inhibit p53 activities in many human cancers. Thus, disrupting the Mdm-p53 interaction to reactivate p53 is a valuable therapeutic strategy for tumor treatment.

Several strategies have been used to develop small molecules to block Mdm2–p53 interaction. Using structure-based design and high-throughput screening, several groups independently developed distinct small molecules including Nutlins (58), benzodiazepinediones (59), and MI-63/MI-219 (refs. 60, 61; Fig. 2) that bind Mdm2 in its p53-binding pocket and disrupt Mdm2–p53 interaction (58–61). Treatment with these small molecules leads to elevated p53 protein levels, induction of p53 target gene expression, decrease in cell proliferation, upregulation of apoptosis, and reduced tumorigenic ability in a variety of tumor cell lines carrying wild-type but not mutant p53 (58–61). Notably, no significant weight loss or any other gross abnormalities is observed in the mice undergoing Nutlin-3 or MI-219 treatment (58, 61). Another small molecule, RITA (Fig. 2), binds the p53 N-terminus instead of Mdm2 and blocks its interaction with Mdm2 (62). Treatment of cells with RITA results in similar effects as treatment with Nutlins or MI-219 (62). A novel strategy using stapled peptides is also applied to block interaction between Mdm2 and p53 (63). These small synthetic peptides, locked into certain folded shapes by an all-hydrocarbon crosslink, are optimized for protein interaction and are resistant to proteolysis (63). Stapled peptides SAH-p53s (Fig. 2) compete with p53 for Mdm2 binding, reactivate p53, and lead to apoptosis in cancer cells with high levels of Mdm2 (63). Yang and colleagues showed that HLI98 (Fig. 2), a family of small molecules inhibiting Mdm2, could bind Mdm2 at the C-terminus and compromise its E3 ubiquitin ligase activity (64). HLI98 treatment suppresses degradation of p53 and induces p53-dependent apoptosis in tumor cells (64). A different small molecule, INI-26854165 (Fig. 2), reactivates p53 by inhibiting Mdm2 E3 activity and inhibits tumor growth in mouse xenografts modeling multiple types of human cancers (65). As p53-independent G2 arrest was observed in tumor cells upon HLI98 treatment (64), small molecules that bind the Mdm2 RING domain may also inhibit p53-independent functions of Mdm2.

The small-molecule inhibitors of Mdm2 described above show very low affinity for Mdm4 binding. But high levels of Mdm4 attenuate the apoptotic response to the small molecules targeting Mdm2–p53 interaction, for example, Nutlin-3a and MI-219 in tumor cells (57, 61, 66, 67). Given the importance of Mdm4 in inhibition of p53 and its presence at high levels in a variety of human tumors such as retinoblastomas and melanomas, efforts have recently been dedicated to the development of Mdm4 inhibitors. A small molecule, SI-172550 (Fig. 2), targets p53–Mdm4 binding (68). A benzofuroxan derivative, XI-006, suppresses the molecule, SJ-172550 (Fig. 2), targets p53–Mdm4 binding and disrupts the Mdm-p53 interaction to reactivate p53 is a valuable therapeutic strategy for tumor treatment.

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their interaction with p53 (67). Treatment of SAH-p53-8 reduces the viability of wild-type p53-carrying cancer cells that also contain high levels of Mdm2 and/or Mdm4 proteins (67). Of note, wild-type p53-carrying cancer cells with high levels of Mdm4 show minimal response to Nutlin-3 treatment (67). Recently, a dual Mdm2/Mdm4 antagonist, RO-5963 (Fig. 2), was identified that blocks p53 interaction with both Mdm2 and Mdm4 by inducing formation of homo- or heterodimers between Mdm proteins through their p53-binding domains (70). In the p53 wild-type, but not mutant tumor cells, the dual antagonist induces apoptotic activity even in the presence of high levels of Mdm4, whereas Nutlin-3a induces a much weaker apoptotic response (70).

To date, a phase I clinical study of JNJ-26854165 (NCT00676910) to determine safety and dosing in patients with advanced stage or refractory solid tumors has been completed. Although JNJ-26854165 was well tolerated in these patients, no objective responses were observed (71). Several phase I studies of a Nutlin family member, RG7112, in patients with liposarcomas (before debulking surgery), solid tumors, hematologic neoplasms, and soft tissue sarcomas (NCT01143740, NCT01164033, NCT00559533, NCT00623870, and NCT01605526) have been completed or are in progress. Acceptable safety profiles and favorable clinical outcomes including complete remission, induction of apoptosis, and decrease in proliferation were seen in patients treated with RG7112 for leukemias, lymphomas, sarcomas, and other solid tumors (72–76). Further studies are needed to verify the efficacy of this agent in long-term cancer treatment.

Despite these initial outcomes, concerns arise for potential adverse effects of Mdm2/Mdm4 inhibitor treatment of patients with cancer. Genetically restoring p53 activity in mice in the absence of Mdm2 leads to ablation of the classically radiosensitive tissues including bone marrow, spleen, thymus, and intestine, which results in 100% fatality of mice within 5 to 6 days (77). In addition, mice heterozygous for Mdm2 or Mdm4, although normal, are sensitive to low-dose IR treatment (78), suggesting that the haploinsufficiency of Mdm2 and Mdm4 in response to DNA damage is detrimental for the organism. These 2 observations question whether p53 activated by Mdm2/Mdm4 inhibitors will be toxic to normal tissues especially under stressed conditions. Xenograft models indicate that the tumor-suppressing doses of Nutlin-3, MI-219, and SAH-p53-8 are well tolerated in mice, leading to no significant weight loss or any gross abnormalities (58, 61, 67). Moreover, the treatment with Nutlin-3 in combination of the therapeutic DNA-damaging agent topotecan results in regression of retinoblastomas without any obvious side effects in mice (56), implying that Mdm protein inhibitors may not be toxic to normal tissues even when combined with other therapeutic agents in patients. However, these data should be cautiously interpreted, as these preclinical studies in mice were short-term, ranging from 6 to 20 days. It still needs to be examined whether prolonged administration of Mdm inhibitors together with other antitumor agents will be detrimental to patients.

Other studies have shown the interaction of Mdm2 with mutant p53 (79, 80). Disruption of this interaction causes stabilization of mutant p53 (79, 80), which has been shown
to have gain-of-function metastatic activities (81). Treatment with Mdm2 inhibitors might lead to unfavorable outcomes in patients with even one allele of mutant p53. Therefore, it is extremely important that Mdm2 inhibitor treatment should be given to patients with tumors carrying only wild-type and not mutant p53. Moreover, Nutlin-3 treatment of SJSA-1 tumor cells (p53 wild-type) leads to selection for p53 mutations and subsequent resistance to Nutlin-3 treatment (82). In addition, selected p53 mutants may lead to more adverse effects, if mutant p53 accumulates and exerts gain-of-function activities in the resistant cells. Thus, combinational therapies and intertreatment evaluation of p53 status should be a standard-of-care for patients with cancer administered Mdm inhibitors. Finally, accumulation of Mdm2 proteins are observed when Mdm2/Mdm4 inhibitors are applied to normal and tumor cells (58–60, 62, 64, 66, 69, 70), and accumulated Mdm2 can execute p53-independent oncogenic functions as discussed in this review. Therefore, administration of Mdm2/Mdm4 inhibitors to patients with cancer might induce oncogenic side effects, which may be evident only after a long latency. Thus, studies with long-term follow-up will be necessary to evaluate the safety of these inhibitors.

Concluding Remarks

Studies of Mdm2 and Mdm4 have long been focused on their roles as the negative regulators of the p53 tumor suppressor. Small molecules and synthetic peptides antagonizing Mdm2 and/or Mdm4 reactivate p53 in preclinical studies and have produced favorable outcomes in patients with cancer in early-phase clinical trials. And yet, accumulating evidence has implicated p53-independent functions of Mdm2 and Mdm4, which accelerate tumorigenesis and tumor progression. However, it remains largely unclear whether inhibiting the binding of Mdm proteins to p53 compromises their p53-independent functions. Therefore, mouse models carrying mutations that will abrogate or enhance the p53-independent functions of Mdm proteins may serve as valuable tools to examine the relationship between these functions and tumor phenotypes. Furthermore, drugs targeting these p53-independent activities of Mdm2 and Mdm4 may potentiate the effectiveness of pharmaceuticals targeting Mdm2 and Mdm4 to reactivate p53 in cancer treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Q. Li

Writing, review, and/or revision of the manuscript: Q. Li, G. Lozano

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References


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