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P53/MDM2 Complex-Based Targeted Strategies in Colon Adenocarcinoma

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Abstract
In the current molecular review, we describe the mechanisms of *TP53/MDM2* deregulation and their impact on the colon adenocarcinoma molecular substrate and phenotype. Among the genes that are critically altered in carcinogenesis, the *TP53* tumor suppressor gene is of major importance. The *TP53* gene (gene locus: 17p13.1) regulates the cell cycle by controlling the G1/S and G2/M checkpoints securing the normal sequence of cell cycle phases. Furthermore, it is involved in apoptosis programmed cell death. The gene is mutated or epigenetically altered in all epithelial malignancies, including colon adenocarcinoma. Additionally, Mouse Double Minute 2 Homolog (*MDM2*), a proto-oncogene (12q14.3), acts as a major negative regulator for p53 expression in the p53-MDM2 auto-regulatory pathway. MDM2 binds directly to p53 and represses its transcriptional activity, promoting p53 degradation. **Conclusion.** In colon adenocarcinoma, *MDM2* oncogene overexpression directly influences p53 oncoprotein expression levels.

Key Words: Colon ▪ Carcinoma ▪ p53-MDM2 ▪ Immunohistochemistry ▪ Genetics.

Introduction

Carcinogenesis is a multiple-step procedure based on a variety of different chromosome and gene imbalances and modifications (1, 2). Gross numerical chromosome alterations, known as Chromosome Instability (CI), include polysomy/aneuploidy and monosomy, whereas point mutations/substitutions, deletions and amplifications comprise specific gene numerical abnormalities, respectively (3). Interestingly, combinations of these genetic alterations lead to aggressive phenotypes in the majority of malignancies (4). Detection and isolation of specific genetic signatures in solid malignancies provide a rational way for oncologists to handle sub-groups of patients on the basis of suitable and relatively efficient, targeted chemotherapeutic regimens (5). *TP53* and *Mouse Double Minute 2 Homolog (MDM2)* are highly significant genes, critically involved in the carcinogenetic process of colon adenocarcinoma (6). They comprise an intracellular complex. Deregulation of the *TP53/MDM2* genes' auto-regulatory pathway is observed in various solid malignancies, including colon adenocarcinoma. Concerning colon adenocarcinoma, the Knudson two-hit hypothesis seems to be perfectly fitted regarding *TP53* suppressor gene inactivation (7).

In the current molecular review, we describe mechanisms and targeted strategies for *TP53/MDM2* deregulation, and their impact on the colon adenocarcinoma molecular substrate and phenotype.

The P53/MDM2 Auto-Regulatory Pathway: Anatomy and Function

The evolution of molecular biology in the past three decades has revealed a galaxy of genes/proteins,

their interactions and functional mechanisms inside the normal or transformed/altered cellular microenvironment (8). Extensive molecular analyses have concluded that *TP53* is a key regulator gene securing genome stability and function, involved in specific signaling transduction pathways, such as p53-sirtuin1 (SIRT1), a conserved nicotinamide adenine dinucleotide (NAD^+) (9). The gene is located on the short (p) arm of chromosome 17 at position 13.1 (17p13.1) and encodes for a nuclear phosphoprotein (molecular mass of 53 kDa). This protein acts as a strong transcription factor that negatively regulates cell proliferation (10). In fact, p53 regulates the cell cycle by causing arrest at stages of the G1/S and G2/M checkpoints (11). This function prevents DNA damage from being inserted in the S phase of DNA replication. Besides its prominent function, *TP53* acts as a positive regulator of histone de-acetylation and apoptosis, and a negative regulator for telomerase activity, proteolysis and helicase activity (12, 13). Additionally, *TP53* is a strong modulator for gene transcription, and is also implicated in biochemical mechanisms including cellular response to hypoxia, response to glucose deficit, protein oligomerization and base-excision repair, even in mitochondrial DNA (14). Interestingly, cell cycle arrest has been noticed as a result of P53-mediated indirect transcriptional repression due to activation of the P53/P21/DREAM/E2F/CHR pathway (15). Concerning P53 protein expression levels, it is expressed in low and moderate levels in normal cells that are visualized by immunohistochemistry assays as a nuclear staining pattern (16). Strong mutated P53 nuclear expression is detected in 50% to 60% of the solid malignancies examined of different histo-genetic origin (17).

MDM2 (also known as E3 ubiquitin-protein ligase) is a proto-oncogene (gene locus: 12q14.3) that encodes a nuclear-localized protein. Enzyme and zinc ion binding to specific intra-cellular substrates and ligase/transferase activity represent the main biochemical MDM2-mediated functions (18). Combined with P53, it forms an auto-regulatory pathway (Figure 1). MDM2 binds directly to p53, acting as a major negative regulator by repressing its transcriptional activity, and promotes p53 proteasomal degradation (19). In fact, MDM2 binds to the N terminus of p53, enhancing p53 ubiquitination and finally degradation. Amplification is the major mechanism of *MDM2* gene transformation to oncogene, and its overexpression in solid malignancies, mainly sarcomas, is frequently associated with more aggressive phenotypes in the corresponding patients (20). *MDM2* mutations have also been reported to impair the ability to degrade P53 oncoprotein efficiently (21, 22).

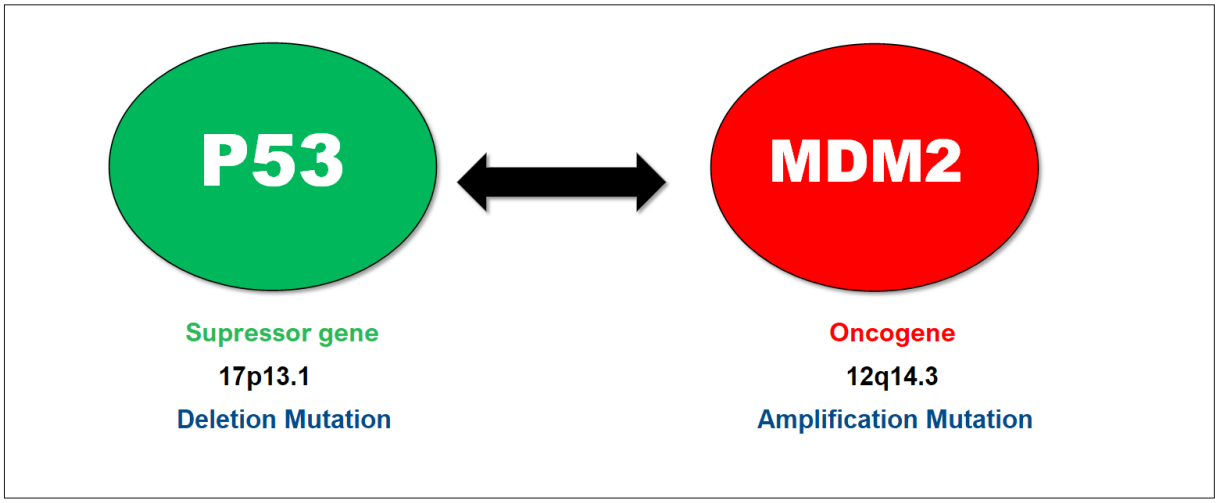

Figure 1. P53 and MDM2 form an auto-regulatory pathway. MDM2 binds directly to p53, acting as a major negative regulator by repressing its transcriptional activity, and promotes p53 proteasomal degradation.

P53/MDM2 Alterations in Colon Adenocarcinoma

Combined normal P53 and MDM2 expression secures cell cycle stability and functionality, partially under the influence of ubiquitin ligases (23). In neoplastic, pre- and malignant tissues this balance is aborted, leading to excessive cell proliferation. Concerning colon adenocarcinoma, there are significant new data based on the influence of MDM2 and also MDM4 on P53 degradation. One study group reported MDM2/MDM4/mitogen-activated protein kinase kinase (MEK) anti-P53 synergistic activity in colon adenocarcinoma. They concluded that nutlin-3, acting as an MDM2-p53 inhibitor, combined or ,not combined with a chimeric small interfering RNA and trametinib, induced activation of wild type *TP53* and simultaneously inhibition of the *KRAS* mutant oncogene (24). In fact, trametinib enhanced G1 phase arrest and promoted induction of apoptotic death. Additionally, another study focused on the causes of increased chemo resistance in sub-groups of colon adenocarcinoma patients. The researchers showed that elevated resistance to paclitaxel, a cytostatic agent combined with nutlin-3a, previously referred to as a P53/MDM2 inhibitor, could be a result of a universal efflux defense mechanism (25). Interestingly, specific peptides, such as PNC-27, seem to be strong agents implicated in P53/MDM2 inhibition in colon adenocarcinoma, destroying colon carcinoma stem cells by blocking the membrane H/MDM-2 (26). In conjunction, another study group explored the role of another agent in P53/MDM2 inhibition. Combined application of the RITA agent with cisplatin in colon adenocarcinoma cell cultures led to P53 activation by suppressing *MDM2* function (27). Furthermore, Tripartite motif-67 (TRIM67), a member of the TRIM protein family responsible for cell cycle regulation (arrest), DNA repair and apoptosis, restores P53 normal expression, thereby sensitizing *in vitro* colon adenocarcinoma cell series to specific chemotherapeutic regimens (28). For this reason, the P53/TRIM67 axis seems to be of significant importance regarding novel targeted therapeutic strategies. Similarly, the HS-1793 resveratrol analog has been found to disrupt the P53-MDM2 complex effectively (29). Another micro-genetic marker, the *lncRNA MIR4435-2* host gene (*MIR4435-2HG*), located on chromosome 2, is implicated in a broad spectrum of intracellular signaling transduction pathways, including Wnt/β-catenin, Hippo, PI3K/AKT/m TOR/PTEN, MAPK/ERK, TGF-β and the P53-MDM2 complex. The marker blocks a series of approximately 20 micro-RNAs and, especially in colon adenocarcinoma, enhances cisplatin activity (30). Similarly, hinokiflavone is a natural biflavonoid promoting pre-mRNA splicing. Interestingly, the agent acts as a potential anti-MDM2 inhibitor, suppressing its mRNA synthesis at the transcriptional level. Concerning colon adenocarcinoma, a study group revealed that the molecule enhanced G2/M phase arrest and apoptosis induction in a series of malignant colon cell cultures, by activating *TP53 gene* in parallel (31).

Multi-target oncoprotein blocking by specific tyrosine kinase inhibitors (TKIs) is a novel, very promising oncological approach in solid malignancies, including colon adenocarcinoma. One study group showed that application of combined selumetinib (a MEK inhibitor) with KRT-232 (a MDM2 inhibitor) *in vivo* in patient-derived xenograft (PDX) colon carcinoma models induced P53 activity, promoting apoptosis (32). Furthermore, the combination of rigosertib and 5-FU in colon adenocarcinoma cell culture-based models positively regulates P53, e-cadherin and CD31 expression, also inhibiting *MDM2* oncogenic activation independently of the presence of *KRAS* mutations (33). Decreased metastatic potential and neo-angiogenesis are the results of rigosertib influence in the corresponding colon cell series. In conjunction, diarylpentanoids act as MDM2/X ligands. In a molecular study, the corresponding researchers investigated their effect on P53-MDM2/X interaction. They observed that diarylpentanoids demonstrated significant anti-proliferative effects in HCT116 cell series (34). Another agent that seems to critically affect the P53-MDM2 pathway is the zinc finger protein SNAI2 (Slug). The molecule increases

MDM2 oncogenic activity, and promotes P53 and P21 cellular expression deficiency by degrading them. A study group analyzing its effect *in vitro* on HCT116 cells showed that a Slug-dependent P53 decrease is an important genetic event that crucially desynchronizes cell cycle phase succession (35). Moreover, DJ-1 has been found to modulate the *TP53/MDM2* signaling pathway by disrupting their interaction and reducing BCL2-, BAX, and CASPASE-3 activity, leading to increased cell proliferation and decreased apoptotic rates (36). This imbalance negatively affects the normal function of the P53/MDM2 complex. Additionally, DJ-1 demonstrates strong oncogenic activity in SW480 and HCT116 malignant cell lines by promoting cell proliferation, invasion and migration. All of these actions are mediated by over activation of the cyclin-D1/MDM2-p53 signaling pathway. In contrast, another agent that seems to affect not only the P53/MDM2 complex but also the PI3K/AKT signaling transduction pathway is costunolide, a natural sesquiterpene lactone. One study group observed that this molecule activated and stabilized P53 by inhibiting its MDM2-mediated ubiquitination, also providing *in vitro* AKT's phosphorylation suppression (37).

Finally, P53/MDM2 involvement in immune response and stromal microenvironment modifications is a very promising field of research in solid malignancies, including colon adenocarcinoma molecular mechanism. Another study group analyzed the potential interactions of the complex with the PD-1/PD-L1 pathway (38). The researchers reported a new mechanism that joins anti-PD-L1 checkpoint blocking immunotherapy strategies with MDM2 inhibitors in patients with normal wild-type P53 expression, claiming a new approach in abnormal intracellular pathway disruption.

Additionally, concerning the clinical relevance of MDM2 in colon adenocarcinoma, small interfering LINC00342 (siLINC00342) was found to be co-overexpressed with MDM2 oncoprotein deregulating the miR-545-5p/MDM2 axis (39). The study group showed that targeting LINC00342, cancerous cell proliferation was decreased, combined with increased apoptotic activity. Furthermore, TP53 mutated protein overexpression is involved in resistance to specific chemotherapeutic-based strategies, including oxaliplatin. An experimental study suggested that targeting aurora-A, a significant kinase in G2/M phase could provide elevated response rates to the corresponding patients with adenocarcinoma (40).

Conclusion

In conclusion, understanding the molecular nature and deregulation mechanisms of the P53/MDM2 complex in solid malignancies, and particularly in colon adenocarcinoma, is a challenge for further investigation. P53 suppressor activity antagonizes MDM2 oncogenic activity in neoplastic and malignantly transformed cells. P53/MDM2 interaction regulates most crucial cell cycle phase successions, and their desynchronization negatively affects the equilibrium between normal cell survival and apoptotic death, leading to the aberrant cell proliferation of malignant cells. Development of targeted anti-MDM2 strategies combined with P53 enhancement should open new horizons in handling colon adenocarcinoma patients rationally on the basis of specific genetic signatures.

Review Highlights

This review study represents a rigid and updated multi-synthesis of all novel molecular knowledge in the field of P53/MDM2 in colon adenocarcinoma, especially focused on modern oncological approaches for targeting this significant autoregulatory pathway for cellular homeostasis.

Conflict of Interest: The authors declare that they have no conflict of interest.

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