

Role of TP53 Gene Mutations in the Pathogenesis of Colorectal Carcinoma

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Abstract

Colorectal cancer (CRC) is multifactorial, and the third most commonly diagnosed cancer in the world. The underlying molecular mechanisms that drive colorectal carcinogenesis are not fully known. Tumor Protein 53 (TP53) gene, the most novel tumor suppressor gene, acts as a major gatekeeper by preserving the genetic composition of a cell and prevents the oncogenic transformation of a cell. However, Protein 53 (P53) gene mutation, is the most reported somatic mutation which drives a phenomenon of colorectal tumorigenesis and is observed in about 50% of colorectal cancer patients. Murine double minute- 2 gene (MDM2) and Cullin-4A (CUL-4A), Tripartite-motif containing protein 67 (TRIM67), micro-RNA (mi- RNA), Kristen Rat Sarcoma 2 viral oncogene homolog (KRAS), Wilms Tumor protein-1 (WT-1) and insulin-like growth factor-1 (IGF-1) are the other markers of colorectal carcinoma. They inhibit the tumor suppressor effect of TP53 and downstream signaling pathways, resulting in carcinogenesis and serves as a biomarker, a prognostic factor, and a therapeutic target of colorectal carcinoma. P53 antibodies produced by colorectal carcinomatous tissue are the markers used for screening and diagnosis. This traditional review is aimed at providing an overview about the genes and genetics involved in p53 mutations and alteration of its function which then act as one of the initial insult in the precursor of cancer transformation in a colorectal cell which eventually leads to full blown disease. Therefore, further studies like clinical trials, cross-sectional and cohort studies is strongly recommended to provide a strong clinical correlation between genetics and disease presentations, with the basic goal to improve the therapeutic approach to colorectal carcinoma.

The direct binding of TRIM 67 at the C terminal of P53 regulates its post-transcriptional modifications. This inhibits MDM2 mediated p53 ubiquitination and proteasomal degradation by destabilizing p53-MDM2 binding at the N terminal [42,46]. Other TRIM proteins regulating P53 activity include TRIM 6, TRIM 13, TRIM 19 who are P53 activators while TRIM 28, TRIM 65 are the P53 inhibitors [47]. In wild-type P53 induced colorectal cancer, TRIM 67 replenishment serves as a useful therapeutic approach, as evident from the above discussion that TRIM 67 loss repress p53 activity and its downstream signaling reactions [48].

TRIM 67 acts as a tumor suppressor protein in colorectal cancer cells and thus any insult to TRIM 67 plays a critical role in colorectal neoplasia development by directly inhibiting p53.

Cullin-4A (CUL-4A) and Protein P53 (p53) expressions in Colorectal Cancer:

Cullin-4 A (CUL-4A) is a protein encoded by the CUL-4A gene. CUL-4A belongs to the Cullin family of ubiquitin ligase E3 proteins, which are significant for the ubiquitination of numerous distinct tumor suppressor genes, like p53, p21, p27 [49]. Although CUL-4A acts as an oncogene, its role in the pathogenesis of colorectal carcinoma is still unclear. Histochemical analysis on tissue microarray (TMA) revealed that expression of both p53 and CUL-4A is found to be significantly raised in colorectal cancer tissue as compared to normal colorectal tissue. Tumor size, differentiation, N stage, tumor invasion, and AJCC stage are associated with high CUL-4A expression in colon cancer.

Key Words

Molecular pathogenesis, p53 oncogene, tumor suppressor gene. colorectal cancer, tp53 mutations, oncogenesis.

Introduction & Background:

"The state of the health of the individual is equivalent to the state to the health of the colon" [1].

Woody Harrelson

Colorectal carcinoma is the third common human cancer, with approximately 1.2 million new cases and 608,700 deaths every year [2]. It is the fourth common cause of cancer-associated mortality in males and third in females [3]. Colorectal cancer patients have a good prognosis in stage 1 and 2 while prognosis and therapeutic response is poor in stage 3 and 4 [4]. Colonic adenocarcinoma is heterogeneous and is characterized by a wide range of mutations, both genomic and epigenomic [5]. Mostly colorectal carcinogenesis results from the inactivation of tumor suppressor genes like Tumor Protein 53 (TP53), Mother against decapentogenic homolog 4 (SMAD4), and Adenomatous Polyposis Coli (APC) as well as activation of oncogenes. In one study, five key mutations including APC, TP53, SMAD4, Kristen Rat Sarcoma 2 viral oncogene homolog (KRAS), and phosphatidylinositol-4,5-Bidphosphate 3-Kinase Catalytic Subunit Alpha (PIK3CA) act via inducing transformation and tumorigenesis [6].

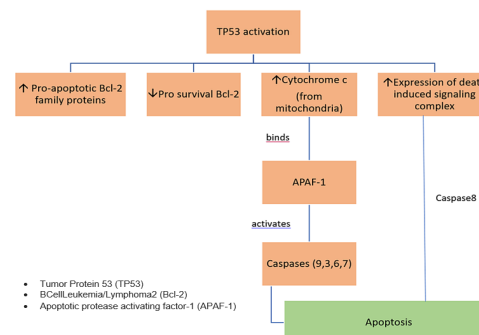
Familial adenomatous polyposis and Lynch syndrome (AD) are the forms of colorectal cancer, which account for 5-15% of all colorectal cancer cases [7]. Loss of wild-type P53 function and RAS activation act synergistically in the malignant transformation of colorectal cells in FAP [8]. High frequency of TP53 mutations is observed in both proximal (34%) and distal (45%) tumors and are also associated with the disease prognosis [9]. Gene expression profiling shows that different genes are expressed differently in the proximal colon and the distal colon [10,11]. Distal colorectal carcinoma mostly shows TP53 mutations, human epidermal growth factor receptor 2 (HER-2) amplification, and epidermal growth factor receptor (EGFR) [11,12].

In ubiquitin ligase E3, CUL-4A is an essential and significant part of the structure, has the ability to degrade many proteins by the ubiquitin-proteasome system. H3K4 methylation by ubiquitin hydrolysis of WDR5 and RBBP5 is regulated by CUL-4A-DDB1 which can lead to activation of ZEB1 and other genes leading to CUL-4A induced EMT (Epithelial-Mesenchymal Transition), tumor migration, invasion, and metastasis of neoplastic cells [50]. Studies have shown that MDM2 and the CUL-4A-DDB1-ROC1 complex regulate the P53 protein levels. Deletion mutations of CUL-4A-DDB1 result in overexpression of P53. However, abnormal supply of CUL4A results in low p53 levels and thus promotion of cancer development [51].

CUL-4A expression is an important predictive factor for colorectal carcinoma. Along with p53, CUL-4A level monitoring in patients with colorectal carcinoma is required for therapeutic and prognostic purposes.

Interaction between Tumor Protein 53 (p53) and micro-RNA (miRNA):

Over the past few years, micro-RNA has been the focus of many studies as these genes are involved in different types of human cancers [52] including colon cancer. miRNA inhibits the tumor suppressor effect of TP53 and downstream signaling pathways, resulting in carcinogenesis. miRNA influences genes controlling apoptosis (TNFRSF10B, PERP, and IGF-1), cell cycle arrest (CCND1, CDK4, CCNB1, CKD1, and GTSE1), angiogenesis inhibitor (THBS1), exosome-mediated secretion (STEAP 3), DNA repair and angiogenesis (RRM2) [53]. miR-150-5p inhibit TP53 in colorectal carcinoma [54]. On activation, TP53 genes regulate both mitochondrial and death receptor pathways of apoptosis and prevent a cell from transforming into a malignant cell [55], as further shown in Figure 2.



TP53 gene mutations are the most reported somatic gene mutations in human carcinomas [13]. Low levels of TP53 expression maintains homeostasis in the cell cycle and cell death. Different stress factors like hypoxia, oxidative free radicals, UV irradiation, and oncogene activation leads to TP53 activation which then goes on to trans-activate or represses downstream genes to regulate apoptosis, cell arrest, angiogenesis, DNA repair, and metastasis [14].

TP53 mutation is observed in nearly 50% of colorectal carcinoma cases. Major genes involved in this pathway include Tumor Protein 53 (TP53), murine double minute- 2 gene (MDM2), tumor protein p73 (TP73), and tumor protein p63 (TP63), which plays an important role in cancer incidence, management, and prognosis [15,16]. P53 is expressed at low levels in a cell through a negative feedback loop of a transcriptional target protein MDM2, which once activated, mediates P53 degradation by binding to ubiquitin and leads to P53 proteasomal degradation. MDMX (MDM4) forms heterodimers with MDM2, increase MDM2 levels to degenerate P53. TP53 gene is located on the short arm of chromosome no 17, has 11 exons and 10 introns. The p53 is a phosphoprotein of 393 amino acids (55kDa) and has an amino-terminal acidic transcription activation domain (1-67), a proline-rich region (67-98), a DNA binding domain (98-303), a nuclear localization signal-containing region (303-323), an oligomerization domain (323-363) and a C terminal basic domain (363-393) [17]. Mostly TP53 mutations are between exons 5 and 8 at codons 175, 245, 248, 273, and 282 [18]. As GOF action of mutant TP53 requires its accumulation in the cell and on immunohistochemistry overexpression of mutant p53 confirms the presence of GOF mutation. However, if no p53 is detected in cancer cells on immunohistochemistry, it shows LOF mutation of TP53 [19-21].

In the light of the aforementioned considerations, in this traditional review, we explore the molecular role of TP53 in the pathogenesis of colorectal carcinoma. This review will be a significant source of information for all the medical students, and it will provide them with knowledge of one of the basic culprits of colorectal carcinoma pathology.

Figure 2: TP53 activation and downstream reactions leading to apoptosis.

TP53 activation leads to increased expression of pro-apoptotic B cell Leukemia/Lymphoma2 (Bcl-2) proteins but decreases the expression of the pro-survival Bcl-2, resulting in decrease permeability of the mitochondrial outer membrane. Caspase 9 becomes reactive when cytochrome C from mitochondria binds to APAF-1, resulting in downstream activation of Caspase -3, -6, -7. Activated p53 form complexes with death-inducing signal receptors to induce apoptosis [56].

The deleterious effect of Tumor Protein 53 (p53) and Kristen Rat Sarcoma 2 viral oncogene homolog (KRAS) in colorectal carcinoma:

KRAS is an important GTPase encoded oncogene whose mutation in colorectal patients is related to poor disease prognosis [57]. Expression of KRAS and p53 in the colon drives the adenoma-carcinoma sequence as demonstrated in Figure 3.

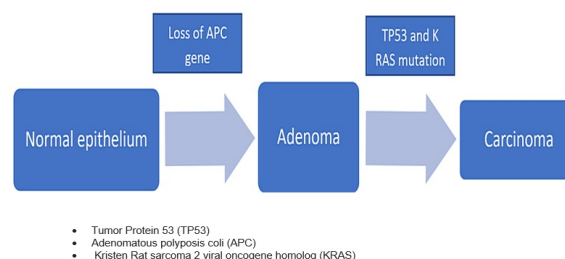


Figure 3: Adenoma carcinoma sequence

According to Fearon and Vogelstein's colon cancer model, in sporadic carcinoma first insult is loss of APC gene, transforming normal mucosa to adenomatous mucosa followed by KRAS and TP53, which converts adenoma to carcinoma [58], as shown above. Concurrent mutations of KRAS and TP53 gene mutations account for >80% of cases of colorectal carcinoma [4]. Most frequently, TP53 mutations occur in exon 5-8 [59] and for KRAS we see most mutations in codon 12, 13 within exon 1 [60]. Combined mutations of KRAS and TP53 are seen in vitro and animal model cancer development [7,61]. These combined mutations favor a trend of extrahepatic metastasis of colorectal carcinoma [62].

Discussion

Colorectal carcinoma is multifactorial [22]. Epidemiological data has divulged that the frequency of colorectal carcinoma is rising in the younger population below fifty years of age [23]. Major factors governing colorectal carcinoma include sedentary lifestyle, lack of physical activity, dietary habits (a diet low in fibers, fruits, and vegetables, and rich in processed food and red meat), Smoking, Alcohol, and Obesity [24,25]. Of the ones listed above, obesity induces insulin resistance leading to hyperglycemia, increases the rate of chronic inflammation commencing from imbalances of an insulin-IGF-1 axis, sex hormones, and adipokines which all come together to increase the risk of colon cancer in obese people [26].

Role of p53 in a cell

p53 is a protein produced by a TP53 gene, the most novel tumor suppressor gene, which acts as a major gatekeeper by preserving the genetic composition of a cell. It has a significant role in the regulation of DNA structure and its stability [9]. p53 modulates cell homeostasis like maintenance of a cell cycle, cell survival, DNA damage repair, free radical removal, immune system regulation, cell proliferation, and apoptosis [21,22,27] and thus prevents the oncogenic transformation of a cell [9].

TP53 gene mutation

TP53 gene mutation incorporates many hot spots and minor sequence variants in its structure [28]. It is the most frequently reported somatic mutation of the human body neoplasias, [27] indicating identical clones of cancer cells. As TP53 is a tumor suppressor gene, therefore, its inactivation, mutation, or loss promotes tumorigenesis [29]. and we associate its presence with tumor occurrence, progression, development, invasion, and metastasis [21,30]. TP53 gene mutations can be either hereditary or sporadic. Germ-line mutations of TP53 in colonic tissue can lead to hereditary cancer predisposition syndrome known as Li-Fraumeni Syndrome [31]. In Li-Fraumeni Syndrome, the most common site of TP53 mutation is the DNA binding domain, which promotes nullification of the sequence-specific DNA binding activity of TP53 resulting in an invasion, metastasis of tumor cells, and cell death inhibition [32].

Hence, TP53 as a tumor suppressor gene and KRAS as an oncogene play a critical role in colorectal adenoma to carcinoma development, and both are used as prognostic and predictive biomarkers of colorectal carcinoma.

Wilms Tumor protein 1 (WT1) and insulin-like growth factor-1 (IGF-1) levels in colorectal carcinoma:

Location of a polyp in the colon influences the WT1 positivity, adenomatous polyp ratio, and a staining score of p53. Therefore, the p53 score, WT1, and IGF1 levels are directly linked to each other. As we know, mutated expression of the tumor suppressor gene (TP53) leads to malignant transformation of normal colonic mucosa to colorectal carcinoma and so are the other important genes, WT1, and IGF1 [63,64]. Studies have demonstrated that WT1 levels correspond to LN metastasis, CRC progression, and staging [65]. In a study conducted by Oji. et al., WT1 levels were raised in colorectal cancer [66]. Similarly, IGF-1, IGF-2, IGF-1R, IGFBP-3 genes were studied by multiple scientists in colorectal carcinoma cells. Zhang et al. [67] Study revealed that mRNA of IGF-1R gene was raised in colon adenocarcinoma [68]. Keku et al. [69] study showed a higher IGF-1R gene level but low mRNA of IGF-1R in colorectal cancer cells. Peter et al. [70] study revealed no association between colorectal IGF-1 gene expression and carcinoma clinical manifestations. However, Shiratsuchi et al. [71] reported that IGF-1 levels have a close association with tumor size, depth, and degree of invasion (vascular and lymphatic both).

WT1 and IGF-1 are the other markers whose levels fluctuate in colorectal carcinoma. Close surveillance of these hormones is required to control colorectal malignancy. However, different studies showed different results, indicating the need for further studies.

P53 antibodies in colorectal carcinoma and their significance:

Tumor markers are the proteins used for screening, diagnosing, monitoring, and determining the prognosis of a disease/cancer. For colorectal carcinoma, CEA and CA 19-9 are the most eminent tumor markers. Anti P53 antibodies are the autoantibodies produced against p53 in colorectal carcinomatous tissue [72].

TP53 mutations can be either GOF or LOF type. GOF mutations result from the binding of p53 to p63, p73 (p53 family proteins) and resulting in their inhibition.

a) By p63 inactivation, mutant TP53 can modulate the expression of pro-invasive transcription proteins, namely, Dicer, Depdc1, Cyclin G2, and Sharp 1 [33].

b) By activating histone modifications, through binding of mutant TP53 with chromatin regulatory genes, namely, methyltransferases MLL1, MLL2, and acetyltransferases MOZ [34].

Many studies have signified the alliance between the mutated TP53 gene and p53 protein expression. In one study of ovarian cancer, they categorized P53 immunoreactivity into three classes:

- 1) <5% positively stained nuclei --- Low p53 expression
- 2) 5-69% positively stained nuclei --- Intermediate p53 expression
- 3) >70% positively stained nuclei --- High p53 expression

Low p53 expression was reported to be linked with non-missense mutations and high p53 expression with missense mutations. However, wild-type TP53 mutations have intermediate p53 expression [35].

From the above discussion, it is evident that P53 has a pivotal role in maintaining the cell's equilibrium. Any insult to this protein whether hereditary or sporadic can lead to molecular and cellular abnormalities, which can then serve as an initial insult in the carcinogenesis pathway.

Mouse double minute homolog (MDM2) and Protein 53 (p53) pathway:

MDM2 homolog, also known as an E3 ubiquitin ligase, is a protein encoded by the MDM2 gene. On binding with p53, MDM2 blocks its activation and thus acts as an oncogene [36]. Overexpression of MDM2 results in low levels of p53 in a cell [37]. MDM2 binds to the p53 and moves it out of the nucleus for ubiquitination and proteasomal degradation (an essential step for inactivation of tumor suppressor function of TP53) [37,38].

As the TP53 gene may have diverse mutations, and so the immune response is produced by these antibodies. Some mutated p53 proteins may even not appear in the serum [73]. Pedersen et al. conducted a retrospective cohort study to demonstrate the significance of p53 antibodies in colorectal carcinoma screening. Results showed elevation of 4 isoforms of p53 protein (p53-9, -10, -25, -78). The most interesting fact is that it reported anti p53 antibody levels to be elevated in blood about 1.4 years before the appearance of clinical manifestations of cancer in these patients [74].

Although p53 antibodies have garnered attention, the significance of anti p53 antibodies in determining the course of the disease along with its severity, the extent of metastasis, invasion, and prognosis has yet to be established.

LIMITATIONS:

I have used the full-text papers published in the last five years to collect my data. I have preferred population who were diagnosed with colorectal cancer after 65 years of age to write the above traditional review. The role of many genes in the development of colorectal carcinoma has not been discussed, with others just their basic introduction is given. Despite these limitations, the expression of mutated TP53 reflects tumor progression in colorectal cells.

Conclusions:

Although there is significant knowledge on TP53 and its role in carcinogenesis, there is much that has yet to be explored. Future studies with a focus on understanding the basic molecular chemistry of p53, potentiality for its use as a biomarker and its association with other biomarkers, and as a possible therapeutic target in the treatment of colorectal carcinoma are vital and recommended. In addition, knowledge of the cellular mechanisms important in the progression of colorectal carcinoma is essential. Advancement in these particular areas is vital as they can give us insights for potential improvements in the current therapeutic approaches to Colorectal carcinoma and all other neoplasms in which TP53 is associated. It is for the above reasons that future clinic trials, cross sectional and cohort studies are strongly recommended.

MDM2 blocks P53 interaction with transcriptional co-activators [39] and promotes its bonding with transcriptional co-repressors [40]. In turn, p53 regulates MDM2 levels by interacting with its promoter region [36,41]. p53 and MDM2 interaction is illustrated below in Figure 1.

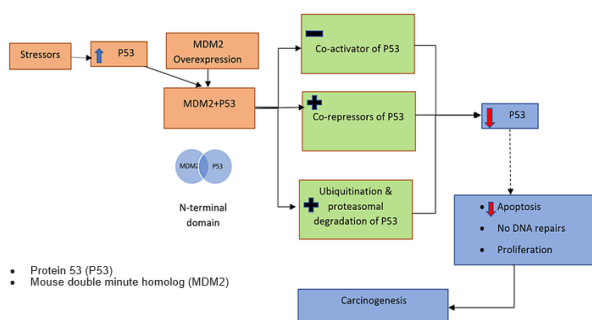


Figure 1: MDM2 and P53 interaction and their downstream regulatory steps

The above autoregulatory pathway helps to maintain low levels of p53 in normal cells, as high levels of this anti-proliferative and pro-apoptotic protein promote tumorigenesis [36]. MDM2 and p53 interact with each other via the N terminal' domain [42]. In the MDM2 structure, the C-terminal RING finger domain act as an E3 ubiquitin ligase and promotes p53 proteolysis at various lysine residues [43,44] by binding to mutant P53 and help to regulate normal activity [45].

Since MDM2 is one of the major regulators of P53, its overexpression in colorectal carcinoma represses Tumor Suppressor Gene (TSG) P53. Strategies to target MDM2-p53 interaction will help to maintain normal p53 levels in a cell and thus cell's homeostasis.

Tripartite-motif containing protein (TRIM) activates Protein P53 (p53) to suppress colorectal cancer initiation and progression:

TRIM67 belongs to the TRIM (tripartite motif) family, is a protein-coding gene. TRIM 67 has a degenerate RING domain in its structure with defective E3 ligase activity [42,46]. TRIM genetically gets mutated in about 80% of colorectal carcinomas and indicates a poor prognosis. Numerous in-vivo and in-vitro studies have confirmed the anti-oncogenic properties of TRIM 67 in colon cancer [9].

In conclusion, the TP53, a novel tumor suppressor gene plays a major role in the pathogenesis of colorectal carcinoma; the associated mutations are sufficient in driving the pathogenesis of colorectal malignancy. TP53 expression in primary colorectal tumors serves as an independent marker for tumor prognosis. The above discussion is valuable for medical students, especially those who are fascinated by genetics, to establish a strong baseline of TP53 gene mutation and its inimical consequences.

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