

Wingless signaling pathway family relation to colon cancer

Have we come full circle?

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ABSTRACT

Colorectal cancer (CRC) is one of the most common malignancies worldwide. Advances in molecular techniques have provided deep insight into the molecular pathogenesis, biologic and genetic changes occurring in colon cancer patients. Current theories of malignant transformation postulate that development of colon cancer is related to 2 main pathways; the loss of heterozygosity pathway, which is usually due to a defect in the adenomatous polyposis coli (APC) gene and microsatellite instability, which is usually due to a defect in mismatch repair (MMR) genes. This review summarizes the role of the wingless signaling pathway genes including APC and MMR genes in the development of CRC.

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Since the original identification of the wingless (Wnt) signaling pathway, questions have been asked about its relevance to human cancer. Intensive molecular studies revealed the involvement of activated Wnt signaling pathways in various human cancers, including those of the colon, liver, endometrium, ovary, prostate and stomach.¹ Genetics studies have identified mutations in key regulators of Wnt/ -catenin pathways in a variety of cancers, most frequently in colon cancer. So it is generally accepted that both dysfunction of the Wnt signaling pathways, including mutations in the adenomatous polyposis coli (APC) and -catenin genes and genetic instability play an important role in colorectal carcinogenesis. Colorectal carcinogenesis is a multi step process when genetic and epigenetic events determine the transition from normal cell to malignant cells. Intensive research has shown that 2 types of colorectal cancers (CRC) follow distinct carcinogenic processes.^{2,3} The first

process called loss of heterozygosity (LOH) is characterized by loss of chromosomal segments bearing tumor suppressor genes. More than two-thirds of sporadic CRCs and familial adenomatous polyposis (FAP) belong to this group. Mutation of the second allele leads to complete inactivation of these genes in the early stage of tumor growth. A hierarchical pattern of loss indicated by inactivation of the APC gene followed by loss of p53 gene is frequently observed.^{4,5} The second process is called microsatellite instability (MSI); it is characterized by genetic instability at microsatellite loci. Approximately 15% of sporadic CRC and more than 90% of hereditary non-polyposis colorectal cancers (HNPCC) belong to this group where the phenotype is a direct result of a defective mismatch repair (MMR) system. Frequently this deficiency is catalyzed by defects in gene hMLH1 or hMSH2, the function of which is silenced by biallelic inactivation (Figure 1).⁶ The

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role of the Wnt has been described in *Drosophila*, *Xenopus* and in vertebrates. This pathway is important in organ development, cellular proliferation, morphology, mortality and the fate of embryonic cells.^{7,9} The Wnt protein activates cell proliferation when it binds to a membrane receptor called Frizzled (Fz) on the cell membrane. This binding sends a signal to a group of proteins within the cell. This group of proteins activates β -catenin (which is the main member in the Wnt signaling pathway) and translocates it to the nucleus where it interacts with another group of proteins called T-cell factor complex (TCF), to continue transcribing, therefore continuing cell proliferation. There is a significant control exerted on the activity of these complexes. Multiple proteins comprise destruction complex function to maintain low steady state levels of β -catenin in the cell, thereby controlling the transcription and proliferation process. This group of proteins include glycogen synthase kinase-3 (GSK-3), axin, APC, and transducin repeat containing protein (TrCP). Regulation of cell proliferation is therefore controlled by the presence or absence of the intracellular protein β -catenin. The presence of Wnt ligand and the absence of its antagonists causes appropriate activation of Wnt signaling pathway where Wnt ligand bind Frizzled (Fz)/low density lipoprotein receptor related protein (LRP) complex, activating the cytoplasmic protein disheveled (Dsh) in *Drosophila* and Dvl in vertebrates. The Dsh/Dvl then inhibits the activity of the multiprotein complex β -catenin-Axin-APC-GSK-3 which targets β -catenin by phosphorylation for degradation by proteasome. The overall result is the accumulation of cytosolic β -catenin. Stabilized β -catenin will then translocate into the nucleus and bind to T-cell factor (Tcf)/Lymphoid enhancer factor (Lef) family leading to transcription of Wnt target gene. While without Wnt ligand, there will be initiation of the β -catenin phosphorylation cascade that is performed by GSK-3. Phosphorylation of β -catenin is then recognized by β -transducin repeat containing protein (β -Trcp) and degraded by the proteasome, reducing the level of cytosolic β -catenin. Inappropriate activation of the Wnt pathway has been recently found to play a role in colon cancer (Figure 2). The Wnt/wingless pathway could be altered in 2 different ways according to whether the cancer cells belong to the group of LOH positive or MSI positive tumors.^{10,11}

A. The Wnt in CRC with LOH. The APC gene is known to function as a tumor suppressor through its involvement in the Wnt/ β -catenin signaling pathway. Mutations in the APC gene on chromosome 5q21 are considered as one of the earliest events in the initiation and progression of

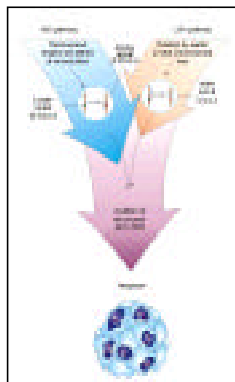


Figure 1 - Schematic illustration of the molecular pathways of colorectal cancer. MSI - Microsatellite instability, LOH - loss of heterozygosity.

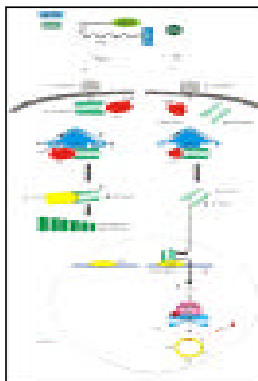


Figure 2 - Schematic presentation of Wnt pathway. a) Wnt pathway in the absence of signal stimulation, b) Inappropriate activation of Wnt signalling pathway in presence of mutant or truncated APC. APC - Adenomatous polyposis coli, Asef - APC-stimulated guanine nucleotide exchange factor, TrCP - Transducin repeat containing protein, Dkk - Dickkopf, Fz - Frizzled, LRP - Low density lipoprotein receptor related protein, MSI - Microsatellite instability, sFRP - Secreted frizzled-related protein, CDK - Cyclin-dependent kinases, Rb - Retinoblastoma protein, TCF - T-cell factor, WIF - inhibitory factor, Wnt - Wingless-type MMTV integration site family member, MMTV - Mouse mammary tumor virus.

CRC. Allelic mutation of APC, whether somatic mutation in sporadic colon cancer or germline mutation in FAP followed by LOH is a common pathogenetic lesion in CRC.^{12,13}

Mutation of APC will activate the Wnt pathway through the following: 1. Activation of β -catenin. The APC gene may act as a negative regulator of β -catenin signaling,^{14,15} as APC protein causes degradation of β -catenin, thus, maintaining low levels of the later in the cytoplasm. Inactivation of the APC gene and consequent loss of APC protein, increases the cellular level of free β -catenin which, in turn, translocates to the nucleus and upregulates cellular proliferation. 2. Disruption of normal cell cycle regulator. Mutation of APC increases the stability and transcriptional activity of β -catenin. The stabilized β -catenin associates with members of the Tcf and the Lef family of transcription factors. There are 4 known members of Tcf and Lef in mammals, one of which Tcf4 is expressed significantly in colon cancer.¹⁴ The β -catenin/Tcf4 complex regulate the proto-oncogene and cell cycle regulator *Cmyc*¹⁶ and *Cyclin D1*.¹⁷ Studies have shown a direct link between the APC gene mutation, β -catenin activation and *Cmyc* up regulation in colon cancer. 3. Disruption of cell-cell adhesion. Interaction of APC with β -catenin and through β -catenin interaction with the members of cadherin family proteins has been implicated in cell-cell adhesion.^{18,19} Cadherin are a family of glycoproteins that act as adhesion molecules between epithelial cells, loss of cadherin can favor the malignant phenotype by allowing easy desegregation of cells, which can then invade locally or metastasize. β -catenin binds to the cytoplasmic aspect of E-cadherin, a cell surface protein that maintains intracellular adhesiveness, so stabilizing the expression of cadherins and cell-cell adhesion.¹⁹ Cancer cells have reduced adhesiveness, resulting possibly from defects in the cadherin-catenin axis. Recent studies^{20,21} showed that interaction of APC with APC stimulated guanine nucleotide exchange factor (ASEF) may regulate the actin cytoskeletal network. Actually, the binding of APC with ASEF controls its activity, therefore, the presence of mutated APC will activate ASEF. The ASEF is activated in CRC cells containing truncated APC. Active ASEF decreases E-cadherin mediated cell-cell adhesion and promotes cell migration. 4. Disruption of normal cell migration. In normal colonic epithelial cells, enterocytes migrate up toward the tips of the villi maintaining the integrity of a light layer of cells with concomitant differentiation. The APC plays an important role in cell migration in maintaining the direction of upward movement of these cells along the crypt-villus axis. Recent findings suggest that mutated APC may be involved in poly p formation

by influencing not only the proliferation and differentiation, but also migration of epithelial cells. So, the loss of APC function will affect the cells and instead of migrating upwards towards the gut lumen, they migrate aberrantly towards the crypt base where they accumulate and form polyps.²²

B. The Wnt in CRC with MSI. Microsatellite instability is characterized by variation of microsatellite sizes in tumor DNA as compared to matching normal DNA due to defects in the MMR. The MMR system is critical for maintaining the genomic stability by serving as a DNA damage surveillance and preventing incorrect base pairing.^{23,24} Defects in the MMR system lead to accumulation of mutations resulting in initiation of cancer. The MSI is present in 15% of sporadic CRC and more than 90% of HNPCC.²⁵ There are at least 6 different proteins required for the complete MMR system. These proteins are hMSH2, hMLH1, hPMS1, hMSH3 and hMSH6 (GTBP). Both mutations and methylation of MMR genes have been linked with MSI playing a causal role in the initiation of CRC.^{26,27} Mutations in the hMLH6 and hPMS2 have been found in approximately 90% of HNPCC cases while mutations in other MMR genes have been less frequent in HNPCC patients.²⁸ Hypermethylation of hMLH1 gene promoter resulting in its transcriptional silencing has been observed more than mutations in sporadic colon cancer. Defective MMR can lead to the MSI of the following target genes:²⁹⁻⁴² a) T-cell factor (Tcf-4).^{30,32,39} b) Transforming growth factor -receptor II (TGF RII).^{29,37,38} c) Insulin like growth factor II receptor (IGFIR).^{31,34,37} d) Phosphatase and tension homologue on chromosome 10 gene (PTEN).^{40,41} e) Caspase 5.⁴² f) The cell cycle regulator E2F4.^{33,36} g) Apoptotic gene Bax.

a) The Tcf-4 gene mutation in MSI. It is known that in Wnt signaling the APC/Tcf-Lef/ β -catenin pathway plays an important role in CRC development. The mutations and their consequences in APC and β -catenin are discussed as characteristic features of type I CRC (LOH CRC). However, Tcf-4 gene mutations are usually found in MSI cells.³⁹ It is expected that mutated Tcf-4 protein may acquire increased transcriptional activity by increased binding affinity for β -catenin or by facilitating the structural organization of chromatin.⁴³

b) The TGF β gene in MSI cells. The binding of Transforming growth factor -receptor (TGF β) to its receptors regulates transcription of growth inhibitory genes. It mediates this effect, in part, by stimulation of cyclin dependent kinase (CDK) inhibitors. These block the cell cycle by inhibiting the actions of cyclin/CDK complexes. So TGF β inhibits cell proliferation by inducing a G1-phase cell cycle arrest acting through increased expression

of p15. Resistance to inhibitory action of TGF has been found in tumor cells in colon cancer which, is due to mutations of the TGF receptor II, its signaling pathway genes and SMAD4, which are tumor suppressor gene products that help to initiate TGF-mediated gene transcription.²⁹ Thus, the loss of TGF IIR or SMAD4 can abolish TGF signaling and stimulate cell proliferation and development of CRC.

c) The IGFIIIR gene mutation in MSI cells. The IGFIIIR performs its growth suppressive function by 2 different mechanisms either it binds and stimulates the plasmin-mediated cleavage and activation of the latent TGF ligands, or it participates in the internalization and degradation of its own ligand IGFII.³⁹ Thus, the loss of IGFIIIR function due to mutations in MSI cells may enhance cell proliferation and therefore, tumorigenesis in colon cancer.^{44,45}

d) The PTEN gene mutation in MSI cells. The PTEN suppressor gene is involved in cell cycle arrest and apoptosis through negatively regulating the survival signaling mediated by phosphatidylinositol 3-phosphate (PIP3) and Kinase (PI3K) and its down stream target, a serine/threonine kinase Akt also called protein Kinase B.^{40,41} Active phosphorylated Akt is involved in cell survival, proliferation and migration.⁴⁶ Loss of inhibitory action of PTEN due to mutation causes elevation of intracellular PIP3 level that stimulates Akt kinase activity and therefore, tumorigenesis.

e) Caspase 5. The caspase 5 gene plays a critical role in mediating apoptosis. This gene contains polynucleotide tracts making it extremely susceptible to shutting/pairing and thus inactivating mutations. Loss of caspase 5 coding potential resulting from instability has been described in lung endometrial and gastrointestinal tumors. It remains to be seen whether the inactivation of caspase 5 prohibits apoptotic signals or modulates inflammatory cytokines⁴² thereby affecting death or survival pathway in colon cancer.

f) The E2F4 gene mutation in MSI cells. The E2F/DP family of transcription factors are required for the regulation of a large number of genes involved in cell proliferation, for example, pRB gene.^{47,48} Defective E2F4 gene expression due to frame shift mutation has been observed in MSI cells in MMR defective colorectal tumors. Therefore, E2F4 mutation in colon cancer cells may promote cell cycle progression.

g) Bax mutation in MSI cells. The proapoptotic and antiapoptotic members of the Bcl2 family play an important role in regulating programmed cell death. The ratio of death antagonists (Bcl2) to agonists (Bax) determines whether a cell will respond to an apoptotic stimulus.³⁹ Approximately 50% of the tumors with MSI are found with mutations in the Bax gene, which will cause increase in the Bcl2-Bax ratio thus resulting in the

escape from apoptosis and inducing colorectal carcinogenesis.

In conclusion, advances in molecular biology studies have led to the identification of 2 different groups of genetic changes that underline CRCs. The first group LOH, which represents 80% of CRCs and is characterized by aneuploidy and allelic losses. The second group of genetic change displays phenotypic MSI (positive tumors), which has a relatively low frequency of allelic losses. A defect in the Wnt signaling pathway has been identified as a major factor responsible for tumor progression in colon cancer. Until now, cancer genotype determination has had no clinical implications. However, the MSI status was recently stressed as a predictive factor for response to chemotherapy.¹⁰ Immunohistochemistry or fluorescent in-situ hybridization analysis using tissue microarray technology (TMA) could represent a complementary strategy to molecular biology in assessing MSI status.^{49,50} The TMA technology could allow us to screen all CRCs for MSI status and may provide valuable management information. Additional molecular studies are required to provide a better understanding of CRC development in our region as most of the molecular information we have is based on United States of America and Western results. Several drugs to reverse the effect of Wnt pathway mutations are under trial, for example, exisulind, which acts as potential cancer therapy through reversing oncogenic APC/ -catenin/Lef/Tcf signals.¹ These advances in targeted chemotherapy highlight the importance of identifying colon cancer genotype.

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