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Future therapeutic implications of new molecular mechanism of colorectal cancer

Lu S *et al*. Novel pathogenesis of CRC

Abstract

High incidence (10.2%) and mortality (9.2%) rates led to the ranking of colorectal cancer (CRC) as the second most malignant tumor spectrum worldwide in 2020. Treatment strategies are becoming highly dependent on the molecular characteristics of CRC. The classical theories accept two models depicting the origin of CRC: The progression of transadenoma to cancer and transformation from serrated polyps to cancer. However, the molecular mechanism of CRC development is very complex. For instance, CRCs originating from laterally spreading tumors (LST) do not adhere to any of these models and exhibit extremely serious progression and poor outcomes. In this article, we present another possible pathway involved in CRC development, particularly from LST, with important molecular characteristics, which would facilitate the design a novel strategy for targeted therapy.

Key Words: Colorectal cancer; Laterally spreading tumors; Molecular mechanism; Truncated adenomatous polyposis coli mutation; Golgi fragmentation; Cancerous mechanism

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Core Tip: Although laterally spreading tumors (LST) are considered vital precancerous lesions of colorectal cancer (CRC), the mechanism mediating their transition to CRC development is unclear. Adenomatous polyposis coli (APC)-truncating mutations driven by Golgi fragmentation are very important cellular events that can abrogate the microtubule binding properties of APC. This effect reduces stability of microtubules, impacts abnormal cell proliferation and survival, causes chromosomal instability, and increases migration. Downstream characteristics of Golgi fragmentation indicate alterations in Ataxia-telangiectasia mutated (ATM) and anoctamin 5 (ANO5)

expression, whereas their gene expression changes are significant in LST. This implies a novel pathway for CRC development from LST.

INTRODUCTION

In 2020, the World Health Organization International Agency for Research on Cancer officially released the latest cancer data, showing a 10.2% and 12.2% incidence of colorectal cancer (CRC)[1,2]. Consequently, CRC was ranked the third and second highest in malignant tumor spectrum and in China, respectively, while its mortality of 9.2% ranked it second highest among all cancers[1,2]. Most incidences of CRC develop from benign polyps (adenomas and serrated polyps) through a series of genetic and epigenetic changes that occur over 10 to 15 years[3,4].

A laterally spreading tumor (LST) is a special digestive tract tumor that is an important precancerous lesion of CRC. Its morphological features are hidden and, consequently, it is easily misdiagnosed. LST can develop into progressive CRC within 3 years with a very poor prognosis[5]. Large-scale controlled studies have shown that LST patients have an 8.4%-52.5% possibility of developing CRC, and benign LST lesions can develop into advanced CRC within 3 years[5].

Pathologically, LST has certain similarities to colorectal polyps and adenoma, and the molecular mechanisms underlying their progression to carcinoma have been clearly elucidated. However, studies on how LST develops into CRC are rare, and the molecular mechanism of the associated carcinogenesis is unclear. Therefore, systematically and comprehensively exploring the molecular mechanisms underlying the malignant transformation of LST to CRC is critical. Specifically, the exploration would have potential theoretical significance and clinical value for early diagnosis and precise treatment of CRC. Here, we present potential alternative pathway mediating the development of CRC, particularly from LST, with critical molecular characteristics, which would facilitate the design of a novel strategy for targeted therapy.

MECHANISM OF CRC DEVELOPMENT

Two classical models have been proposed for the development and progression of CRC from colon epithelial cells. The first model describes the process of transformation of transadenoma to cancer, mainly initiated by mutations in the adenomatous polyposis coli (*APC*) gene. In this model, *APC* mutation is followed by mutations in Kirsten rat sarcoma viral oncogene homolog (*KRAS*)/neuroblastoma rat sarcoma viral oncogenes, mothers against decapentaplegic homolog 4, and finally, tumor protein p53 (*TP53)*.

The other model describes the transformation from serrated polyps to cancer, with the driver mutation of catenin beta 1 secondary to mutations of *KRAS*/B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha[6]. These mutations eventually result in transforming growth factor-beta receptor type 2 (TGFRB2) overexpression[6]. The main signaling pathways involved are Wnt, mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/Akt, and TP53, which are hyperactivated[7]. Several other signaling pathways, including hedgehog, erb-b2 receptor tyrosine kinase, ras homolog family member A, Notch, bone morphogenetic protein, Hippo, AMP-activated protein kinase, nuclear factor kB, and Jun N terminal kinase, also participate in the occurrence and development of CRC[8].

TRUNCATED *APC* MUTATION

Several studies have indicated that *APC* mutations are extremely important in both the mutation frequency and different stages of CRC tumor development[9-11]. APC is the "gatekeeper" gene of the colorectal mucosal epithelium and the key molecule regulating colon epithelial cell homeostasis, polarity, and movement. *APC* acts as a tumor suppressor gene of CRC and 80% of sporadic CRCs harbor mutations, which are widely considered an early event in colorectal malignancy. Somatic point mutations of *APC* mainly occur in the mutation cluster region (MCR); however, *APC* contains several other mutations in its protein-coding region[9,10].

The most important cytological events after mutation in the MCR region are the structural truncation of APC (amino acid sequence from 1362 to 1540, namely the APC-

2,3 repeat, 23) and lack of axin, -catenin, microtubules, and other binding sites[11]. Mutations in truncated APC result in the loss of the microtubule-binding properties of APC and further reduce microtubule stability. Truncating mutations cause *APC* to lose the properties of normal tumor suppressor genes and show "acquired" proto-oncogene properties, including abnormal cell proliferation and survival, chromosomal instability, and increased migration.

Truncated APC binds to and activates APC-stimulated guanine nucleotide exchange factor (ASEF), which is closely related to actin remodeling and movement, which causes significant changes in cell structure and function[12]. Knockdown of *ASEF* or *APC*-truncating mutations markedly reduces cell migration; however, overexpression of full-length APC does not increase ASEF-mediated cell migration. The Golgi complex is a dynamic organelle that is essential for sorting, processing, and transporting proteins by which the stability of cellular structures is maintained.

Fragmentation of the Golgi apparatus is observed in age-related diseases including Alzheimer's disease, Parkinson's disease, and cancer[13]. The Golgi apparatus may be closely involved in the development of human diseases; however, the mechanisms and significance of its fragmentation are poorly understood. *APC*-truncating mutations induce fragmentation of the Golgi apparatus and lead to structural reorganization of cytoskeletal proteins and actin[13]. This causes cells to exhibit abnormal biological behavior, such as loss of polarity and increased migration[13]. Therefore, *APC*-truncating mutations driven by Golgi fragmentation are an important event in normal cells.

Mutation-rich regions of *APC* in the normal mucosa, LST, colorectal adenocarcinoma, and colorectal adenoma specimens were detected using polymerase chain reaction-single-strand conformation polymorphism[14]. The results of that study showed that while no *APC* mutations were observed in the normal mucosa of the large intestine, the mutation rates were 25%, 30%, and 27.8% in LST, colorectal adenocarcinoma, and colorectal adenoma, respectively[14]. The difference in rates between the specimens was not statistically significant and the results were consistent with other reported *APC* mutation rates of 15.5%-42.4% in LST specimens. Two recent studies showed *APC*

mutation frequencies of 80% (10 samples) and 57% (14 samples) in LST[15]. Therefore, *APC* mutation may act as an important initiator in LST development[16].

TP53* AND *CRC

TP53 and *CRC* are important intracellular tumor suppressor genes, and the main biological function of *TP53* is the repair of cellular damage. Normal *TP53* can be used to monitor the integrity of genomic DNA in real time. During DNA damage, TP53 stops cell division at the G1/S phase to allow cells enough time to repair the damage. For irreparable DNA damage, TP53 induces programmed apoptotic cell death, thereby inhibiting the generation of possible mutant cancerous cells[17,18].

TP53 mutations primarily occur during the middle and late stages of carcinogenesis. Numerous *TP53* mutations reduce the proportion of wild-type *TP53* and weaken its function in monitoring genomic DNA integrity, thereby allowing tumorigenesis[19]. Studies on *TP53* and *LST* are scarce, a recent comprehensive and unbiased screening of the genome, epigenome, and transcriptome was conducted based on the Cancer Genome Atlas (TCGA) database[14].

Bioinformatic data integrated from 11 LST samples and validated in an additional cohort of 84 benign colorectal injury samples, identified several high-frequency genetic, epigenetic, and transcriptional alterations[14]. Deletions occurred in chromosomes 1p, 5q, 14q, and 18, whereas doubling occurred in chromosomes 7, 8, 13, 19, and 20. Furthermore, these alterations were highly prevalent in the panel of the colorectal and rectal adenocarcinoma validation groups. The main signaling pathways associated with LST are axonal guidance, thyroid cancer, human embryonic stem cells pluripotency [Nanog homeobox (NANOG)], and Wnt/β-catenin.

Cohort validation studies compared 10 LSTs with 212 CRC samples with a focus on five major altered signaling pathways, Wnt, TGF β, PI3K, MAPK, and P53[20,21]. The results showed that the differences in the TGFβ and TP53 signaling pathways were significant[20,21]. The results suggested that ataxia-telangiectasia mutated (ATM), a very important molecule in the TP53 pathway, was significantly increased, which stabilized

TP53 molecules. The expression of nother very important gene, anoctamin 5 (*ANO5*), was significantly reduced, leading to mitochondrial fragmentation.

Our results based on TCGA data analysis also confirmed a significantly low expression level of *ANO5* in LST. The difference in the expression of the Golgi fragmentation-related genes *ATM* and *ANO5* was significantly different. This differential expression weakened the expression of the wild-type TP53 transcription factors MDM2 proto-oncogene (MDM2), TP73, and subsequently modulated TP53 stability in the TP53 signaling pathway. These two important signaling molecules are closely related to the stability of TP53 mutants. These data suggest that TP53 mutation has a unique molecular mechanism that differs from that in polyps and adenomas in CRC development from LST.

In our opinion, the progression of LST to CRC is distinct from the development of common polyps or adenoma carcinogenesis. Therefore, we proposed a two-stage cascade mutation hypothesis from LST to CRC (Figure 1): The driver stage: APC-truncating mutations drive Golgi fragmentation, resulting in reorganization of cellular structural proteins, thereby leading to abnormal polarity and lateral growth. Cancerous stage: Golgi fragmentation further affects the repair mechanism of TP53 base mismatch mediated by the heat shock proteins MDM2, TP63, and TP73, resulting in increased stability of mutant TP53 and promotion of LST carcinoma.

SPECIAL GENE EXPRESSION ANALYSIS

Based on the mRNA data of 25711 samples from 980 healthy donors in the genotype-tissue expression (GTEx) v8 database, pan-cancer mRNA-sequencing (mRNA-seq, n = 11057) and survival (n = 10121) data were downloaded from TCGA database. We then performed a differential expression analysis of *ATM*, *ANO5*, *APC*, and *TP53* in pan-cancer and adjacent normal samples. Survival analysis of these genes was also performed in pan-cancers.

We analyzed the expression profiles of *ATM*, *ANO5*, *APC*, and *TP53* in human normal and pan-cancer samples and found that *ATM*, *ANO5*, and *APC* were

significantly downregulated in rectal adenocarcinoma esophageal carcinoma (READ), colon adenocarcinoma (COAD), and READ-COAD samples. In contrast, TP53 showed an obvious higher level in READ, COAD, and READ-COAD samples than that in normal samples.

Furthermore, the survival analysis results indicated that *ATM*, *ANO5*, *APC*, and *TP53* expression were correlated with the overall survival (OS) of lung squamous cell carcinoma (LUSC), thyroid carcinoma (THCA), mesothelioma (MESO), pancreatic adenocarcinoma (PAAD), COAD, brain lower grade glioma (LGG), and breast invasive carcinoma (BRCA) patients.

Analysis of ATM, ANO5, APC, and TP53 expression in normal and pan-cancer samples

We analyzed the expression profiles of *ATM*, *ANO5*, *APC*, and *TP53* in normal human tissues based on tropomyosin (*TPM*) gene expression values from the GTEx v8 database. The results indicated that *ATM*, *ANO5*, *APC*, and *TP53* were highly or moderately expressed in most organs or tissues ($1 <$ average TPM < 32), except that *ANO5* expression was low in the whole blood, vagina, and breast (average TPM < 1, Figure 2A).

Then, we performed a differential expression analysis of mRNAs for these genes across 18 cancer types that had over five pairs of cancer-adjacent samples based on the $\log_2$[fragments per kilobase of exon per million mapped fragments (FPKM) + 1] data from TCGA. Figure 2B [heatmap of $\log_2$(FPKM + 1) shows the expression status of *ATM, ANO5, APC*, and *TP53* between cancer and cancer-adjacent samples where red and blue represent upregulation and downregulation, respectively]. Figure 2C shows the $\log_2$(FPKM + 1) expression status of *ATM, ANO5, APC*, and *TP53* between cancer and cancer-adjacent samples.

Table 1 shows that the expression of *TP53* was significantly upregulated in bladder cancer (BLCA), cholangiocarcinoma (CHOL), COAD, ESCA, GBM, KIRC, KIRP, LIHC, LUAD, LUSC, PRAD, READ, STAD, THCA, and UCEC and downregulated in KICH, compared with levels of cancer-adjacent samples ($|\log_2 FC| > 0.19$, $4.99E\text{-}12 < P < 0.03$).

The *ANO5* level was significantly higher in KICH and obviously lower in BLCA, BRCA, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LUAD, LUSC, PRAD, READ, STAD, THCA, and UCEC than it was in cancer-adjacent samples ($|\log_2 FC| > 0.46$, 2.36E-26 < P < 0.003).

APC expression was significantly upregulated in CHOL and LIHC relative to that in cancer-adjacent samples and an obvious downregulation was noticed in BLCA, BRCA, COAD, GBM, HNSC, KICH, KIRC, KIRP, LUAD, LUSC, PRAD, READ, THCA, and UCEC ($|\log_2 FC| > 0.25$, 1.46E-15 < P < 0.03). In addition, the *ATM* expression was obviously upregulated in CHOL, KIRC, LIHC, and STAD compared to that of cancer-adjacent samples. In contrast, BLCA, BRCA, KICH, LUSC, PRAD, THCA, and UCEC ($|\log_2 FC| > 0.12$, 2.14E-16 < P < 0.049) were obvious downregulated.

We also analyzed the difference in expression among COAD, READ, and their cancer-adjacent samples based on the merged and batch-normalized TPM expression data of the GTEx and TCGA. The results showedd that *ATM, ANO5*, and *APC* were significantly downregulated, whereas TP53 showed an obviously higher expression in READ (Figure 2D), COAD (Figure 2E), and READ-COAD (Figure 2F) samples than it did in normal samples ($P < 0.001$).

Association of ATM, ANO5, APC, and TP53 with cancer prognosis

To explore the role of *ATM, ANO5, APC*, and *TP53* in pan-cancer prognosis, we conducted a survival analysis in pan-cancers based on the $\log_2(FPKM + 1)$ data and clinical survival data of 33 cancer types. The survival map of *ATM, ANO5, APC*, and *TP53* expression in pan-cancers indicated that the expression of these four genes was correlated with OS in LUSC, THCA, MESO, PAAD, COAD, LGG, and BRCA (Figure 3A). The relationship among *ATM, ANO5, APC*, and *TP53* and the OS of cancer patients ($^aP < 0.05$, $^bP < 0.01$, and $^cP < 0.001$) was identified.

Briefly, LUSC or THCA patients with *ANO5* expression had a poor OS [1.40 < hazard ratio (HR) < 3.80, $P = 0.014$], whereas MESO or PAAD patients with *ANO5* expression showed a good OS (0.53 < HR < 0.57, 0.0031 < P < 0.018). *ATM* expression was

indicative of a poor OS in COAD (HR = 1.70, *P* = 0.038). *TP53* expression was positively associated with poor OS in BRCA (HR = 1.40, *P* = 0.038), LGG (HR = 1.60, *P* = 0.0067), and PAAD (HR = 12, *P* = 0.0033). In contrast, *ATM* expression exhibited a positive association with good OS in COAD (HR = 0.54, *P* = 0.012). Moreover, *APC* expression showed a good co-relation with OS in BRCA (HR = 0.49, *P* = 9E-06, Figure 3B).

CONCLUSION

APC-truncating mutations driven by Golgi fragmentation are a very important cellular event, which can cause loss of the microtubule binding properties of APC. This effect further reduces microtubule stability, resulting in abnormal cell proliferation and survival, chromosomal instability, and increased migration. Downstream characteristics of Golgi fragmentation include gene expression alterations with *ATM* upregulation and *ANO5* downregulation, which are significant in LST. These observations suggests the existence of a unique and novel pathway for the development of CRC from LST, and future studied of potential CRC treatment should focus on this newly identified mechanism.

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