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ORIGINAL ARTICLE

Basic Study

Alternative splicing of *VEGFA*, *APP* and *NUMB* genes in colorectal cancer

Yi-Jun Zhao, Hua-Zhong Han, Yong Liang, Chen-Zhang Shi, Qing-Chao Zhu, Jun Yang

Yi-Jun Zhao, Hua-Zhong Han, Chen-Zhang Shi, Qing-Chao Zhu, Jun Yang, Department of Surgery, Shanghai Jiao Tong University, Affiliated Sixth People's Hospital, Shanghai 200233, China
Yong Liang, Department of Gastrointestinal Surgery, the Affiliated Hospital of Xuzhou Medical College, Xuzhou 221006, Jiangsu Province, China

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Correspondence to: Jun Yang, MD, Associate Professor, Department of Surgery, Shanghai Jiao Tong University, Affiliated Sixth People's Hospital, No. 600 Yishan Road, Shanghai 200233, China. yangjun_sh@163.com
Telephone: +86-21-64369181
Fax: +86-21-64368920

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Abstract

AIM: To investigate alternative splicing in vascular endothelial growth factor A (*VEGFA*), amyloid beta precursor protein (*APP*), and Numb homolog (*NUMB*) in colorectal cancer (CRC).

METHODS: Real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and PCR-restriction fragment length polymorphism analyses were performed to detect the expression of *VEGFA*, *APP*, and *NUMB* mRNA in 20 CRC tissues and matched adjacent normal tissues, as well as their alternative splicing variants.

RESULTS: qRT-PCR analysis revealed that the expression of *APP*, *NUMB*, and *VEGFA*165b mRNA were significantly downregulated, while *VEGFA* mRNA was upregulated, in CRC tissues (all $P < 0.05$). PCR-restriction fragment length polymorphism analysis revealed that the expression of *VEGFA*165a/b in CRC tissues was significantly higher than in adjacent normal tissues ($P < 0.05$). Compared with adjacent normal tissues, the expression of *NUMB*-PRR^{S} in CRC tissues was significantly decreased ($P < 0.05$), and the expression of *NUMB*-PRR^{L} was increased ($P < 0.05$).

CONCLUSION: Alternative splicing of *VEGFA*, *APP*, and *NUMB* may regulate the development of CRC, and represent new targets for its diagnosis, prognosis, and treatment.

Key words: Alternative splicing; Amyloid beta precursor protein; Colorectal cancer; Numb homolog; Vascular

endothelial growth factor A

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Core tip: This study was undertaken to investigate the effect of alternative splicing of vascular endothelial growth factor A (*VEGFA*), amyloid beta precursor protein (*APP*), and Numb homolog (*NUMB*) genes in colorectal cancer (CRC). We demonstrated that these genes and their alternative splice variants were different in CRC tissues and matched adjacent normal tissues using quantitative reverse transcriptase PCR and PCR-restriction fragment length polymorphism analyses. We conclude that alternative splicing of *VEGFA*, *APP*, and *NUMB* may be associated with the diagnosis, prognosis, and treatment of CRC. This study may help in understanding the relationship between alternative splicing and CRC.

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INTRODUCTION

Alternative splicing (AS), through which different mRNA variants are produced from the splicing of a single gene[1], is a pivotal step in the generation of proteomic and functional diversity. At least 95% of human genes are predicted to generate alternatively spliced transcripts[2,3]. AS is performed by the spliceosome, which is composed of four small nuclear ribonucleoproteins (snRNPs; U1, U2, U4/U6, and U5) and a number of non-snRNP auxiliary proteins. There are several basic patterns of AS: exon skipping (cassette alternative exon), intron retention, mutually exclusive, alternative 5'SS, alternative 3'SS, alternative initiation sites, and alternative polyadenylation sites. AS is regulated by the interaction between cisregulatory sequences and transacting factors. Cis-regulatory sequences include exonic and intronic splicing enhancers, as well as exonic and intronic splicing silencers. Trans-acting factors function by binding to splicing enhancers and silencers and include members of Ser/Argrich and heterogeneous nuclear (hn) RNP protein families. Ser/Argrich protein families are generally considered positive regulators of AS, but not all of them[4]. Similarly, not all hnRNP protein family members are negative regulators[5]. Previous research has demonstrated that AS and the RNA binding proteins and other factors which regulate this process are often disordered in human diseases, including cancers, spinal muscular atrophy, tauopathies, Hutchinson-Gilford progeria syndrome, hypercholesterolemia, familial dysautonomia, and frontotemporal lobar degeneration[6-14].

Due to its roles in promoting proliferation[15], increasing microvascular permeability[16], and inducing neovascularization, vascular endothelial growth factor A (VEGFA) is always associated with cancers, and is involved in tumor vascularity, metastasis development, and recurrence. Similarly, the expression of amyloid beta precursor protein (APP) is correlated with cell adhesion, motility, and proliferation. In addition, Numb (*Drosophila melanogaster*) homolog (NUMB) is a membrane-associated protein that plays critical roles in asymmetric cell division and is regarded as a determinant of cell fate. Previous studies have used AS microarray profiling and reverse transcriptase (RT)-PCR assays to detect enormously profiled alternative exons and demonstrated the relationship between the AS of *VEGFA*, *APP*, and *NUMB* genes and cancer, especially lung, breast, and colon tumors[10]. However, the definite mechanisms of AS underlying these genes have not been fully elucidated.

Colorectal cancer (CRC) is the third most common cancer and the third leading cause of cancer death in men and women[17]. Due to the improvement in living standards and changes in diet structure, CRC morbidity and mortality rates have increased rapidly year by year. Although the exact etiology and mechanism of CRC is unclear, high-risk factors, such as a higher intake of meat, fat, calcium, and vitamin D and lower intake of fiber, fruit, and vegetables, are associated with CRC[18]. Early stage CRC almost always has no symptoms and is difficult to diagnose accurately. When symptoms and metastasis appear, treatment becomes more difficult, and a complete cure may be impossible. Thus, improved efficiency in early diagnosis and treatment is crucial.

In this study, we performed quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and PCR-restriction fragment length polymorphism (RFLP) analyses to determine AS changes in matched normal and tumor tissues from CRC patients. Our results reveal that AS of *VEGFA*, *APP,* and *NUMB* were closely related to CRC. These findings provide evidence that AS may contribute to the development of CRC and these markers may have potential clinical value.

MATERIALS AND METHODS

Patient samples

Matched pairs of primary tumor and adjacent normal tissue samples were obtained from 40 patients with CRC who underwent resection at the Department of Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital from 2012 through 2014. Six cases had synchronous hepatic metastases; the primary tumor and hepatic tumors were simultaneously resected during surgery. Postoperative pathology confirmed that all CRC and hepatic metastases samples were tubular adenocarcinoma. The pathologic stages, depth, histology, and lymphatic invasion of

Table 1 Patient characteristics

Characteristics	*n* (%)
Sex	
Male	21 (52.5)
Female	19 (47.5)
Primary tumor location	
Ascending colon	5 (12.5)
Transverse colon	7 (17.5)
Sigmoid colon	15 (37.5)
Rectum	13 (32.5)
TNM stage	
Ⅰ	7 (17.5)
Ⅱ	14 (35.0)
Ⅲ	13 (32.5)
Ⅳ	6 (15.0)
T factor	
T1	3 (7.5)
T2	7 (17.5)
T3	11 (27.5)
T4	19 (47.5)
Lymphatic invasion	
N0	19 (47.5)
N1	9 (22.5)
N2	12 (30.0)
Distant metastasis	
M0	34 (85.0)
M1	6 (15.0)

TNM: Tumor-node-metastasis.

the primary tumors are shown in Table 1. None of the patients had received preoperative radiotherapy or chemotherapy. All tissue samples were flash frozen within 30 min of surgical removal and stored at -80 ℃ until further use.

RNA extraction and qRT-PCR

We performed qRT-PCR to estimate the expression of *VEGFA*, *APP*, *NUMB*, and *VEGFA*165b. The mucosal scrapes (30 mg) were homogenized for RNA extraction. RNA was extracted using a total RNA extraction kit (SLNCO, China), followed by RT using a qPCR-RT kit (Toyobo Co., Ltd., Osaka, Japan). cDNA was then evaluated by qRT-PCR using Real-Time PCR Master Mix (Toyobo Co., Ltd.) in a FTC-3000 PCR Cycler (Funglyn Biotech, Inc., Scarborough, ON, Canada) using the primers (Generay Biotech Co., Ltd., Shanghai, China) listed in Table 2. Denaturation, annealing, and extension temperatures were set at 94 ℃, 61 ℃, and 72 ℃ for 30 s each, respectively, for 40 cycles according to routine procedures.

PCR-RFLP

PCR-RFLP analysis was performed to detect the expression of *VEGFA*, *APP*, and *NUMB* AS variants. RNA extracted from mucosal tissues served as a template for amplification of these genes using the total RNA extraction kit (SLNCO, China). The RT reaction using the ReverTra Ace qPCR RT kit (Toyobo Co., Ltd.) contained 8 μL H_2O, 6 μL total RNA, 4 μL 5× RT buffer, 1 μL enzyme mix, and 1 μL RT primer, and was reacted at 42 ℃ for 18 min following inactivation of reverse transcriptase at 98 ℃ for 5 min. The PCR reaction included 7.2 μL RNase-free H_2O, 10 μL 2× Taq Master Mix, 0.4 μL forward primer, 0.4 μL reverse primer, and 2 μL cDNA. The 5′ ends of the forward primers (Generay Biotech Co., Ltd.) were labeled with the fluorescent molecule, 6-carboxyfluorescein, listed in Table 3. The annealing temperatures of *APP*, *NUMB*, and *VEGFA* were 55 ℃, 57 ℃, and 58 ℃, respectively. Denaturation and extension temperatures were set at 94 ℃ for 90 s and 72 ℃ for 60 s, respectively, for 38 cycles according to routine procedures. Amplified PCR products were verified by electrophoresis using a 1.5% agarose gel and detected by capillary electrophoresis (ABI3730XL sequencer, Applied Biosystems of Thermo Fisher Scientific, Waltham, MA, United States). The fragment peak size, area, and height were determined by GeneMapper (Applied Biosystems).

Statistical analysis

Real-time qRT-PCR assays were performed in triplicate. Data are presented as the mean ± SE for three or more independent experiments. The differences in *APP*, *NUMB*, and *VEGFA* mRNA levels and their AS variants, between tumor and adjacent normal tissue were compared using a paired *t* test. Differences in mRNA expression levels in tissues inside and outside the serosal layer, tissues with and without lymph node metastasis, tissues in Tumor-node-metastasis (TNM) stages were compared by monofactor ANOVA analysis (GraphPad version 5.01, GraphPad Software, Inc., La Jolla, CA, United States). $P < 0.05$ was considered statistically significant. The statistical methods of this study were reviewed by Yi-Jun Zhao, Hua-Zhong Han, Chen-Zhang Shi, Qing-Chao Zhu, and Jun Yang from Department of Surgery, Sixth People's Hospital affiliated to Shanghai Jiao Tong University.

RESULTS

Different expression levels of VEGFA, APP, and NUMB mRNA between tumor and adjacent normal tissues

Compared with controls, the relative quantitative value of *VEGFA* mRNA was significantly higher in CRC tissues ($P < 0.01$), and the expression of *APP* and *NUMB* mRNAs were significantly lower ($P < 0.01$). The expression of *VEGFA*, *APP*, and *NUMB* mRNA was not correlated with the depth of tumor infiltration, the presence of lymph node metastasis, or the TNM stage (Figures 1, 2 and 3).

Expression of VEGFA AS variants

VEGFA amplified gene fragments of 206 bp, 338 bp, and 410 bp represent *VEGFA*121a/b, *VEGFA*165a/b, and *VEGFA*206a/b, respectively. PCR-RFLP analysis revealed that the expression of *VEGFA* AS variant *VEGFA*165a/b in CRC tissues was significantly higher than that in adjacent normal tissues ($P < 0.05$), the expression of *VEGFA* AS variant *VEGFA*121a/b was not significantly different between CRC tissues and

Table 2 Primers for quantitative reverse transcription-polymerase chain reaction

Gene	Primers	Sequence (5'-3')	Products (bp)
GAPDH	Forward	TGAAGGTCGGAGTCAACGGA	225
	Reverse	CCTGGAAGATGGTGATGGGAT	
VEGFA	Forward	TTGCTGCTCTACCTCCACCAT	270
	Reverse	GGTGATGTTGGACTCCTCAGTG	
APP	Forward	GGCGGAGCAGACACAGACTA	131
	Reverse	ACCTCATCACCATCCTCATCGT	
NUMB	Forward	GCTGGATCTGTCACTGCTTCAT	118
	Reverse	CCACATTCCTTCTCCCGCTTC	
*VEGFA*165b	Forward	TCAGAGCGGAGAAAGCATTTGT	130
	Reverse	TCCTGGTGAGAGATCTGCAAGT	

Table 3 Primers for polymerase chain reaction-restriction fragment length polymorphism

Gene	Primers	Sequence (5'-3')	Products (bp)
GAPDH	Forward	FAM-TGAAGGTCGGAGTCAACGGA	225
	Reverse	CCTGGAAGATGGTGATGGGAT	
VEGFA	Forward	FAM-TGAGCTTCCTACAGCACAAC	206/338/410
	Reverse	TCGATGGTGATGGTGTGGTG	
APP	Forward	FAM-CCTACGAAGAAGCCACAGAG	162/330/387
	Reverse	GGGCATGTTCATTCTCATCC	
NUMB	Forward	FAM-TGCTCCGATGACCAAACCAG	157/301
	Reverse	CACCTCTTCTAACCATCGGTC	

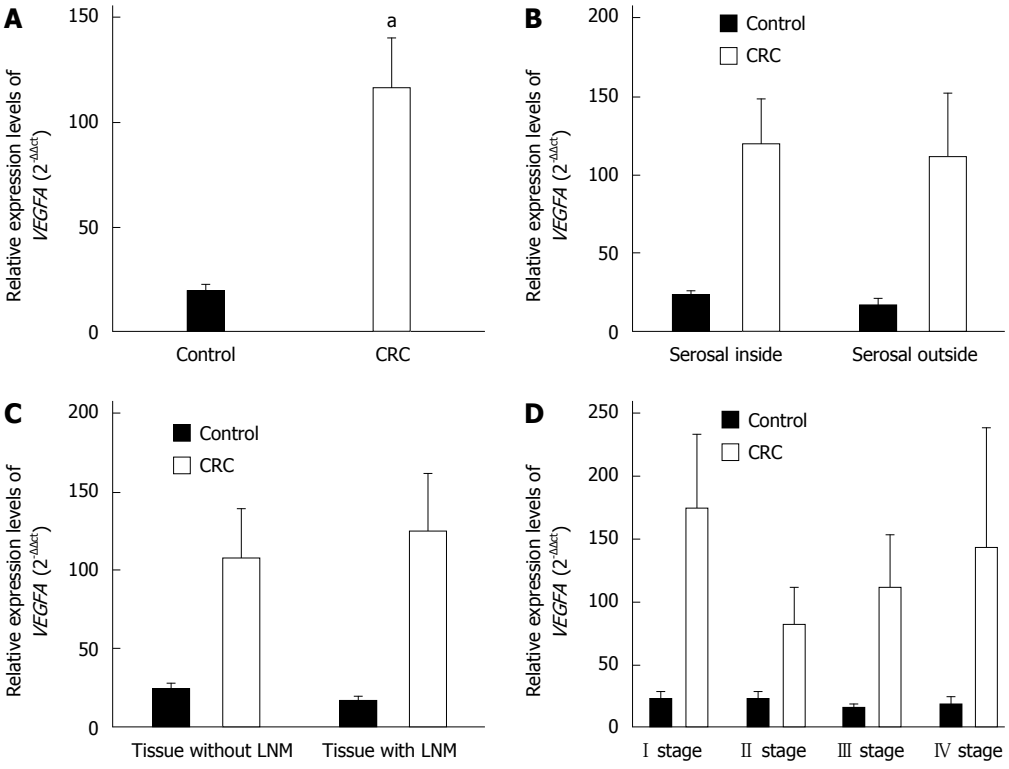

Figure 1 Expression levels of *VEGFA* mRNA assessed by quantitative reverse transcription-polymerase chain reaction. A: Colorectal cancer (CRC) tissues and normal intestinal mucosa tissues; B: Tissues inside and outside the serosal layer; C: Tissues with and without lymph node metastasis (LNM); D: Tissues of tumor-node-metastasis (TNM) stages. $^{a}P < 0.05$ *vs* control.

adjacent normal tissues, and the expression of *VEGFA* AS variant *VEGFA*206a/b was only found in a few samples (Figures 4 and 5).

Expression of APP AS variants

APP generated amplicons of 162 bp, 330 bp, and 387 bp represent *APP*770, *APP*751, and *APP*695,

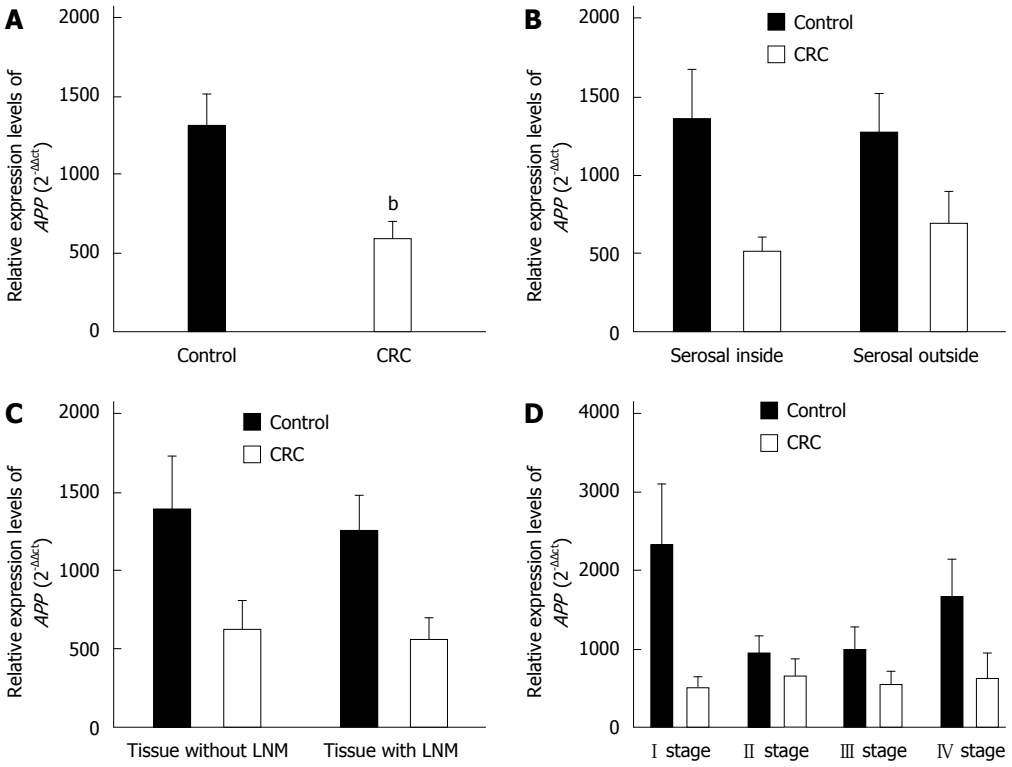

Figure 2 Expression levels of *APP* mRNA assessed by quantitative reverse transcription-polymerase chain reaction. A: Colorectal cancer (CRC) tissues and normal intestinal mucosa tissues; B: Tissues inside and outside the serosal layer; C: Tissues with and without lymph node metastasis (LNM); D: Tissues of tumor-node-metastasis (TNM) stages. $^{b}P < 0.01$ *vs* control.

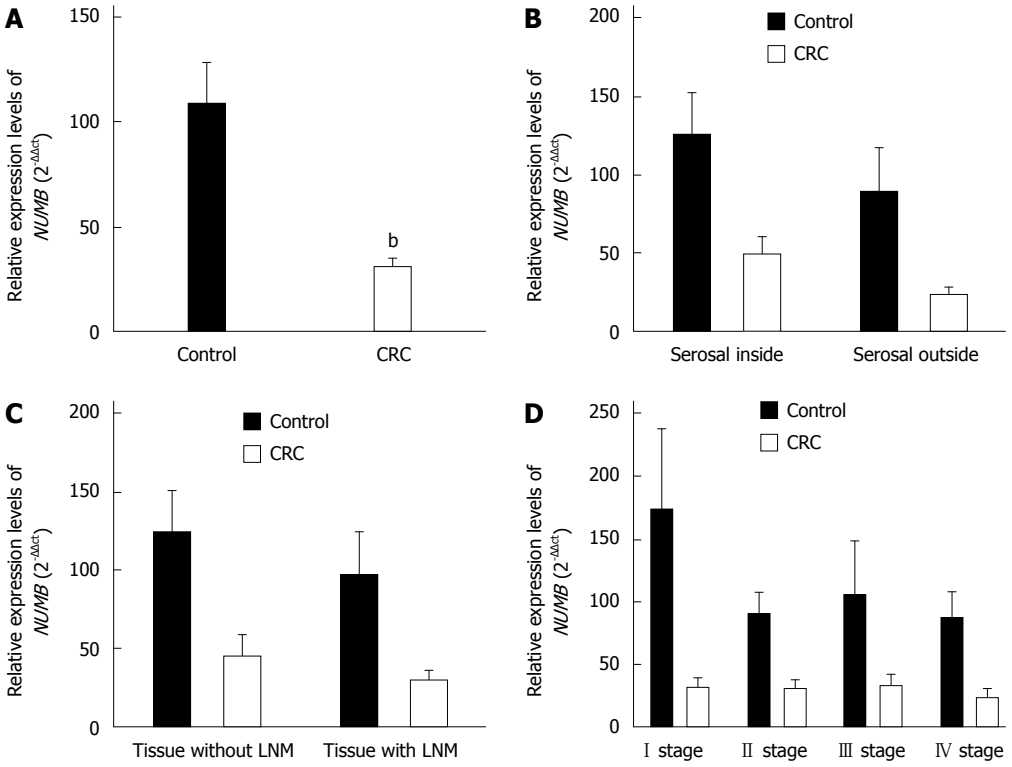

Figure 3 Expression levels of *NUMB* mRNA assessed by quantitative reverse transcription-polymerase chain reaction. A: Colorectal cancer (CRC) tissues and normal intestinal mucosa tissues; B: Tissues inside and outside the serosal layer; C: Tissues with and without lymph node metastasis (LNM); D: Tissues of tumor-node-metastasis (TNM) stages. $^{b}P < 0.01$ *vs* control.

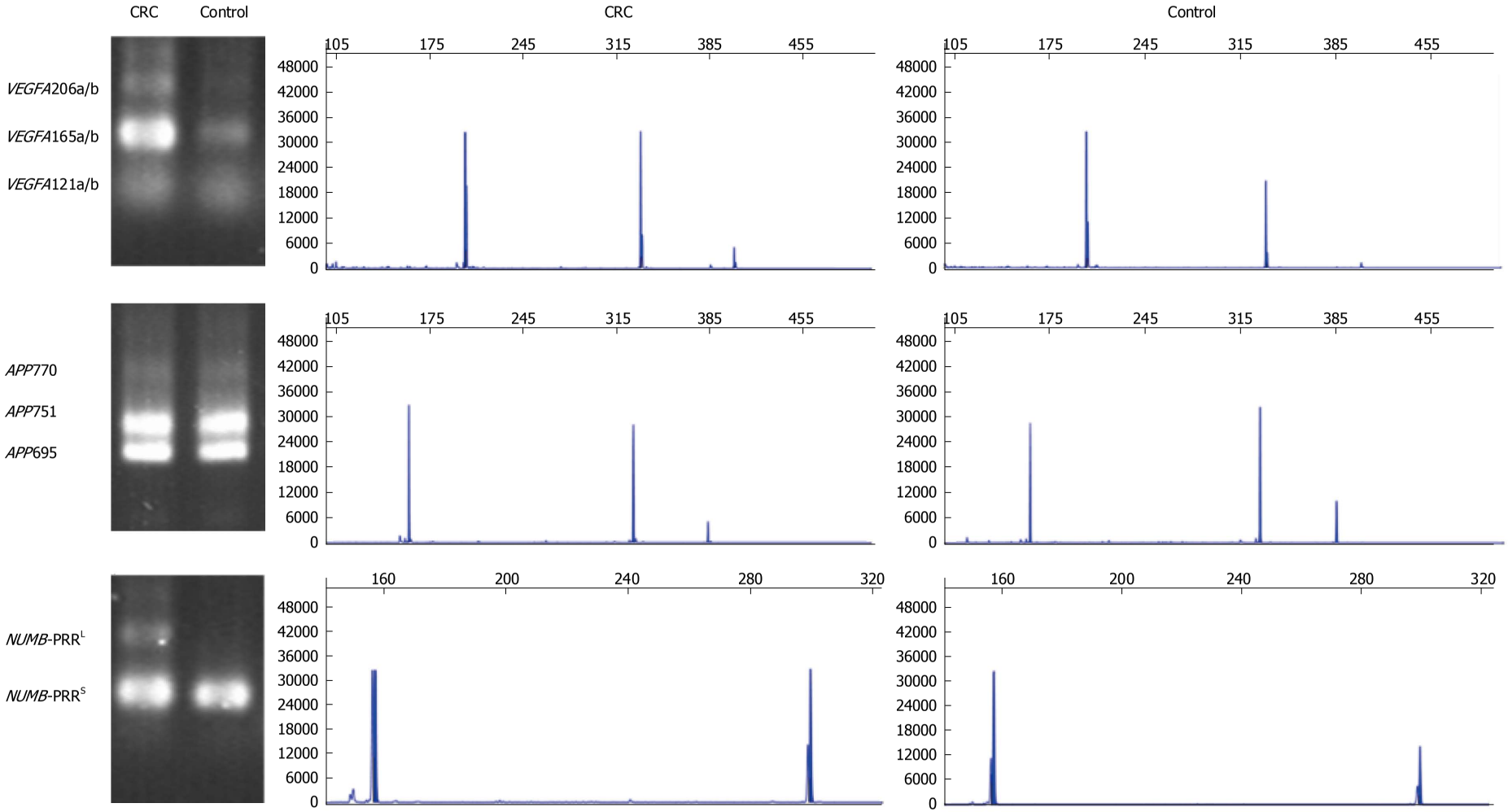

Figure 4 Electrophoregram of alternative splicing variants in *VEGFA*, *APP* and *NUMB*. CRC: Colorectal cancer.

respectively. We detected three *APP* AS variants named *APP*695, *APP*751 and *APP*770, which were not significantly different between CRC tissues and adjacent normal tissues (Figures 4 and 5).

Expression of NUMB AS variants

The products of 157 bp and 301 bp represent *NUMB*-PRR^S and *NUMB*-PRR^L. The expression of *NUMB* AS variant *NUMB*-PRR^S in CRC tissues was significantly lower than that in normal intestinal mucosa tissues ($P < 0.05$), and the expression of *NUMB* AS variant *NUMB*-PRR^L in CRC tissues was significantly higher than that in normal

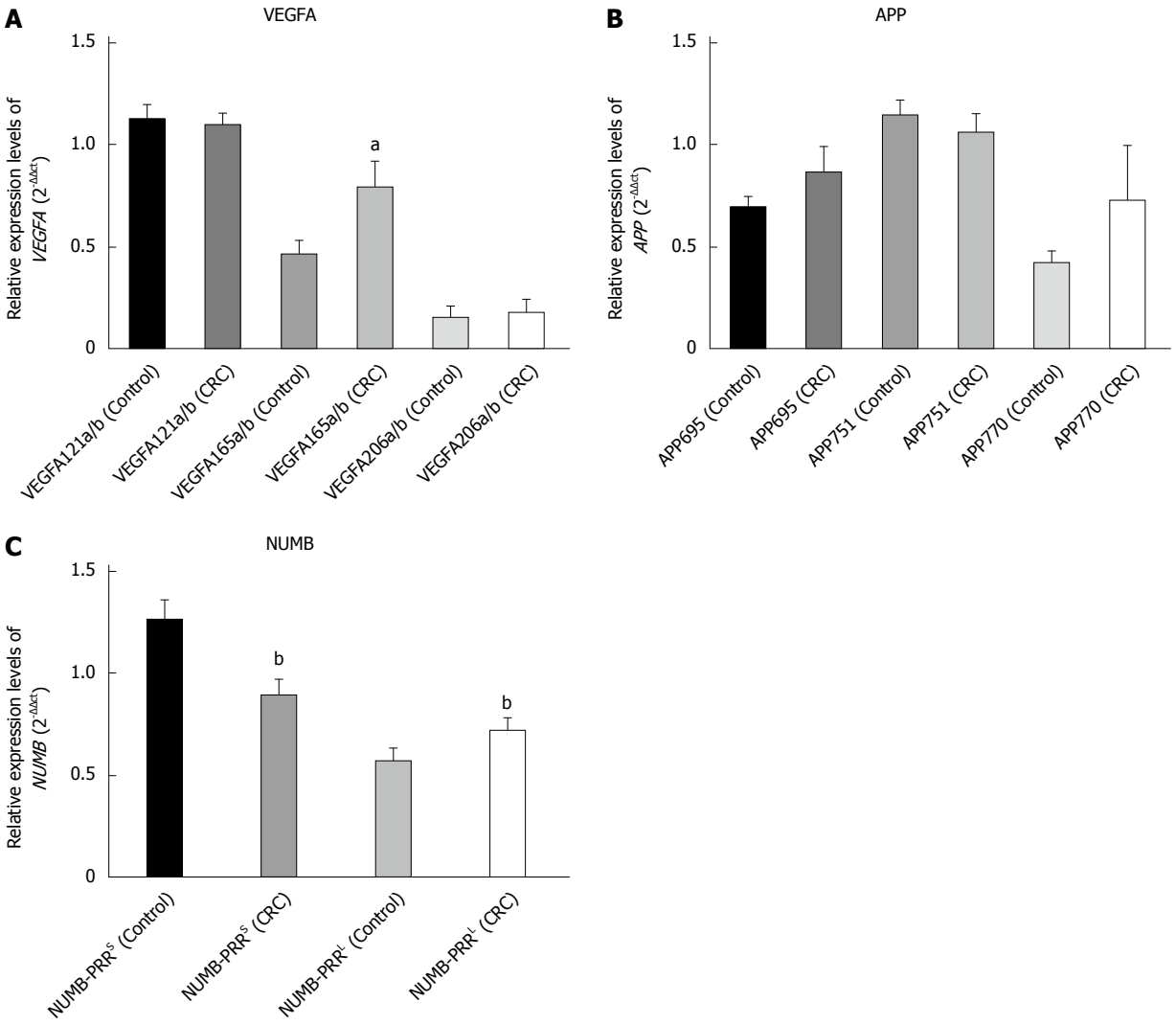

Figure 5 Expression levels of alternative splice variants using polymerase chain reaction-restriction fragment length polymorphism analysis. Expression levels of A: *VEGFA*; B: *APP*; and C: *NUMB* alternative splice variants in colorectal cancer (CRC) tissues and normal intestinal mucosa tissues. $^{a}P < 0.05$ and $^{b}P < 0.01$ *vs* the control group.

intestinal mucosa tissues ($P < 0.05$) (Figures 4 and 5).

Decreased VEGFA165b expression in CRC tissues

qRT-PCR analysis revealed that the expression of *VEGFA*l65b in CRC tissues was significantly lower than that in normal intestinal mucosa tissues ($P < 0.05$). The expression of *VEGFA*165b was not correlated with depth of tumor infiltration, the presence of lymph node metastasis, or TNM stage (Figure 6).

DISCUSSION

Previous studies have confirmed that the expression of *VEGFA* correlates with vascularity, metastasis, and proliferation of CRC[19]. The *VEGFA* gene consists of eight exons separated by seven introns, including three alternative exons (6, 7 and 8), and thus is composed of multiple isoforms. Due to alternative selection of the 3′ splice site in exon 8 resulting in a six amino acid substitution (Cys-Asp-Lys-Pro-Arg-Arg to Ser-Leu-Thr-Arg-Lys-Asp), *VEGFA* isoforms are classified into *VEGFA*xxx (pro-angiogenic) and *VEGFA*xxxb (anti-angiogenic), where xxx denotes the amino acid number[20]. AS of *VEGFA* exons 6 and 7 generates isoforms including *VEGFA*121a/b, *VEGFA*165a/b, and *VEGFA*206a/b. Cheung *et al*[21] found that *VEGFA*121a/b and *VEGFA*165a/b represented a proportion of the total *VEGFA* in both lung and colon samples including normal and tumor tissues, however, *VEGFA*206a/b was not detected in any samples. The protein product of *VEGFA*121a/b is unable to bind to heparin, whereas *VEGFA*165a/b and *VEGFA*206a/b are heparin-binding isoforms[22,23]. As previous studies demonstrated that *VEGFA*121a/b, *VEGFA*165a/b, and *VEGFA*206a/b are closely associated with melanoma[24,25], lung cancer[26,27], colon cancer[21], breast cancer[28], ovarian cancer[29], prostate cancer[30], and bladder cancer[31], our study mainly focused on the expression of these isoforms in CRC. Based on the results of qRT-PCR and PCR-RFLP analyses, we found significant upregulation of *VEGFA* in CRC tissues, with *VEGFA*121a/b and *VEGFA*165a/b being the predominant isoforms, and

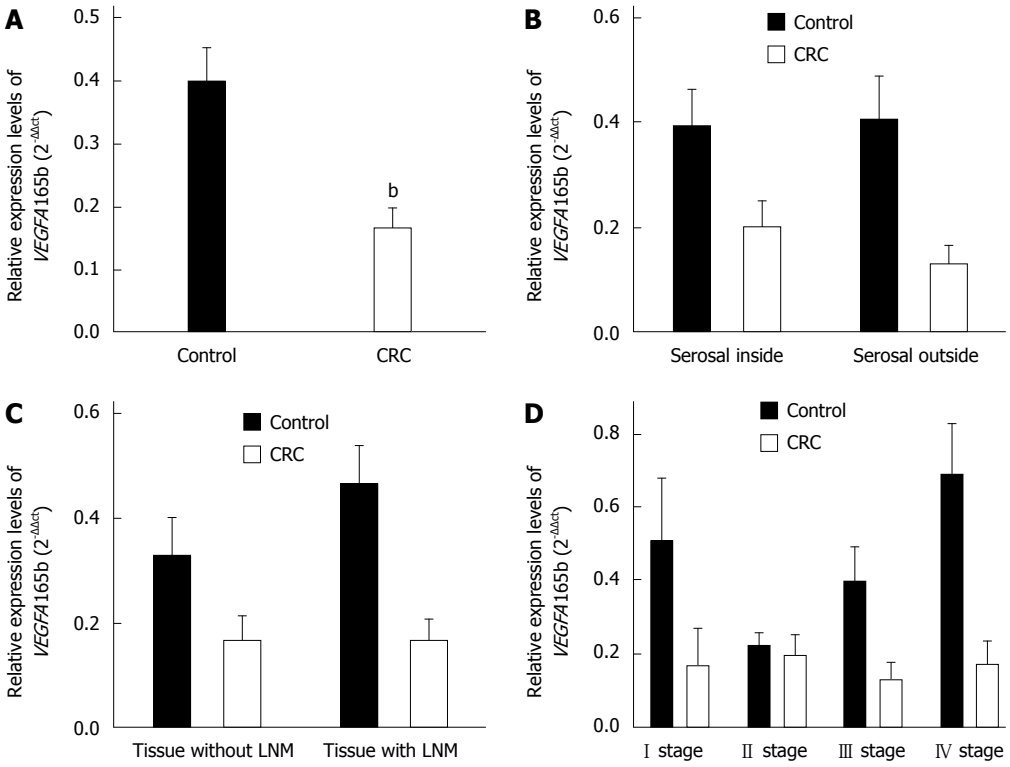

Figure 6 Expression levels of *VEGFA*165b assessed by quantitative reverse transcription-polymerase chain reaction. Expression levels of *VEGFA*165b in A: Colorectal cancer (CRC) tissues and normal intestinal mucosa tissues; B: Tissues inside and outside the serosal layer; C: Tissues with and without lymph node metastasis (LNM); and D: Tissues of tumor-node-metastasis (TNM) stages. $^{b}P < 0.01$ *vs* control.

*VEGFA*206a/b only detected in a few samples. The upregulation of *VEGFA* and *VEGFA*165a/b in CRC tissues is suggestive of their role in promoting the development of CRC. Furthermore, we determined the expression of AS isoform *VEGFA*165b and found that it was downregulated in CRC tissues using qRT-PCR. However, the expressions of *VEGFA* and *VEGFA*165b were not significantly associated with depth of infiltration, lymphatic invasion, or TNM stage, which was likely due to an insufficient number of samples. *VEGFA*165b functions as a tumor suppressor of CRC. Due to AS of exon 8, the residual two arginines of *VEGFA*165b are substituted by lysine and aspartic acid, resulting in loss of one disulfide bond and the inability of *VEGFA*165b to bind the vascular endothelial growth factor receptor 2 and neuropilin 1. The properties of *VEGFA*165b generate inefficient autophosphorylation of the receptor, and account for a series of changes in the downstream pathway. With regard to the major anti-angiogenic isoform, *VEGFA*165b was identified in diverse normal and abnormal human tissues including various tumors[32-35]. It was previously reported that *VEGFA*165b is downregulated in several human cancers, and its overexpression delayed the growth of these cancers[32,35-37]. Recombinant human *VEGFA*165b (rhVEGF165b) treatment *in vivo* has an inhibitory effect on growth; consequently, *VEGFA*165b is deemed a potential anti-cancer target. In addition, the *VEGFA*xxx/*VEGFA*xxxb ratio affects the sensitivity of tumors to bevacizumab, and *VEGFA*xxxb can inhibit the effect of bevacizumab by competitive binding[35]. Hence, bevacizumab may be a feasible treatment by increasing the amount of *VEGFA*xxxb or decreasing the amount of *VEGFA*xxx. In brief, the differential expression of VEGFA isoforms, including *VEGF*165a and *VEGF*165b in CRC, appears to be a possible prognostic factor and potential target in the treatment of CRC.

APP is a Type I integral and widely expressed membrane protein that normally functions in neuroprotection and neurite outgrowth[38]. As a result, APP is considered a crucial step in the molecular cascade of events resulting in the pathogenesis of Alzheimer's disease[39]. Previous research has demonstrated that the expression of APP is associated with cell adhesion, motility, and proliferation. Increased expression in prostate, pancreatic, thyroid, and oral squamous cell cancers showed that APP promotes the growth of these cancers[40-43]. In our research, the expression of *APP* decreased in CRC tissues, which indicates that APP may be involved in the regulation of proliferation and invasion. The reduced expression of *APP* leads to increased activity of tyrosine kinases, a change in p53-associated cell apoptosis signaling pathways, reduced cell adhesion, and increased liquidity, which are linked to cell proliferation and tumorigenesis[44,45]. The differential expression of *APP* was the result of the properties of the cancers

compared to CRC. However, the expression of *APP* was not significantly associated with depth of infiltration, lymphatic invasion, or TNM stage, likely due to an insufficient number of samples. *APP* contains 19 exons separated by 18 introns and encodes different isoforms according to differential splicing of alternative exons 7, 8, and 15[46]. Due to the AS of exons 7 and 8, *APP* can be spliced to produce isoforms such as *APP*695 (skipped exons 7 and 8), *APP*751 (skipped exon 8), and *APP*770 (full-length). The 751 and 770 isoforms contain a 56-amino acid Kunitz-type protease inhibitor domain, whereas *APP*695 excludes this region. Ko *et al*[41] demonstrated that *APP*770 and *APP*751, but not *APP*695, were upregulated in oral keratinocytes and oral squamous cell carcinoma. Our results showed that *APP*695, *APP*751, and *APP*770 were not significantly different between CRC tissues and adjacent normal tissues, likely due to an insufficient number of samples. Consequently, the relationship between the isoforms of *APP* and CRC still need to be determined in the future.

NUMB contains an amino-terminal phosphotyrosine-binding (PTB) domain and C-terminal proline-rich (PRR) and Eps15 homology regions. Related research has shown that *NUMB* was involved in inhibiting the development of cancer through the suppression of epithelial-mesenchymal transition, and Notch and Hedgehog pathways, as well as activation of the p53 gene[47-50]. In our research, the expression of *NUMB* in CRC tissues was significantly lower than that in adjacent normal intestinal mucosa tissues, which suggests that *NUMB* may have a role in inhibiting the development of CRC. However, the expression of *NUMB* was not significantly associated with depth of infiltration, lymphatic invasion, or TNM stage, due to an insufficient number of samples. Due to the AS of exons 3 and 9, generating two splicing variant sites in the PTB and PRR domains, *NUMB* gives rise to four alternatively spliced isoforms, $PTB^{L}PRR^{L}$ (p72), $PTB^{S}PRR^{L}$ (p71), $PTB^{L}PRR^{S}$ (p66), and $PTB^{S}PRR^{S}$ (p65). Previous studies have provided evidence that exon 3 is not significantly different between patient tumor and normal samples in non-small cell lung cancer[10]. Thus, our research focused mainly on the AS of exon 9, which is the distinction between *NUMB*-PRR^{L} ($PTB^{L}PRR^{L}$ and $PTB^{S}PRR^{L}$, exon 9-included) and *NUMB*-PRR^{S} ($PTB^{L}PRR^{S}$ and $PTB^{S}PRR^{S}$, exon 9-skipped). Our results show that *NUMB*-PRR^{L} splice variants are specifically expressed at elevated levels in CRC tissues. In contrast, the expression of the *NUMB*-PRR^{S} splice variant was reduced in CRC tissues. Combined with the increase in *NUMB* expression in CRC tissues, we suggest that *NUMB*-PRR^{S} may have a role in inhibiting the development of CRC, while *NUMB*-PRR^{L} may have a role in promoting the development of CRC. *NUMB*-PRR^{S} appears to be more functional than *NUMB*-PRR^{L}, and consequently, *NUMB* mRNA plays a role in inhibiting the development of CRC. Therefore, the PRR region encoded by exon 9 is closely correlated with the development of CRC. The increased level of *NUMB*-PRR^{L} results in reduced levels of overall NUMB with subsequent Notch activation. It is also possible that *NUMB*-PRR^{L} plays a dominant role in suppressing the Notch inhibitory activity of *NUM*B-PRR^{S}[10]. It has been reported that increased levels of *NUMB*-PRR^{L} may promote cell proliferation, whereas *NUMB*-PRR^{S} mainly has an important role in promoting cell differentiation[51,52]. A study has provided evidence that the splicing regulators, Nova and Fox2, act in a dominant manner in the regulation of *NUM*B exon 9 AS[13,53]. However, the mechanism is unclear, and further studies are essential.

In summary, AS in *VEGFA*, *APP*, and *NUMB* is closely associated with CRC, however, most cancer-associated AS events have not been functionally characterized on any level, and many others remain to be detected. APP functions as a tumor suppressor at the integral level, whereas VEGFA and NUMB play crucial roles in the development of cancer at the integral and individual level. The AS in *VEGFA*, *APP*, and *NUMB* probably provides a possible prognostic factor and potential therapeutic target for CRC. Investigations of these genes at the protein level and their mechanism of action in CRC are urgently needed.

COMMENTS

Background

Alternative splicing (AS) is a pivotal step in the generation of proteomic and functional diversity. Previous research has provided evidence that AS and the RNA binding proteins and other factors, which regulate this process, are often disordered in cancers and other human diseases. Although the link between AS and human diseases has been established, there is little understanding of the effects of AS in the development of colorectal cancer (CRC). Therefore, this study was undertaken to explore the effect of AS of vascular endothelial growth factor A (*VEGFA*), amyloid beta (A4) precursor protein (*APP*) and the Numb (*Drosophila melanogaster*) homolog (*NUMB*) genes in CRC.

Research frontiers

AS is an important post-transcriptional regulatory mechanism and it has been reported to be associated with human disease. The current research hotspots are the exact biologic function, precise regulation and mechanism of AS in human disease, as well as the effects of signal pathways, and the factors in human disease and gene therapies that are based on AS.

Innovations and breakthroughs

Previous research has provided evidence that AS is associated with human diseases, however, there is still little understanding concerning the effects of AS in the development of CRC. In this study, molecular biologic technology was used to detect the expression of *VEGFA*, *APP*, and *NUMB* in CRC, as well as their AS variants. The authors demonstrated the important role of AS in regulating the expression of *VEGFA*, *APP*, and *NUMB*. These findings are crucial in providing gene therapy strategies for CRC.

Applications

This study demonstrates that AS of *VEGFA*, *APP*, and *NUMB* is closely associated with CRC and could be used to provide possible prognostic factors and potential therapeutic targets for CRC.

Terminology

AS, through which diverse mRNA variants are produced from the splicing of a single gene, is a pivotal step in the generation of proteomic and functional diversity. Regarded as an important regulatory mechanism between transcription and translation, AS affects nearly 95% of mammalian genes and multiple regulatory processes. The process of AS is performed by the spliceosome and regulated by the interaction between cisregulatory sequences and transacting factors. Aberrant regulation of AS results in multiple human diseases, including various cancers.

Peer-review

This is a good study in which authors investigated the relationship between alternative splicing of *VEGFA, APP,* and *NUMB* and CRC. The results are reliable and suggest that there is a significant role of alternative splicing of *VEGFA, APP,* and *NUMB* in the development of CRC. This study represents a further step in understanding the mechanisms involved in this relationship.

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