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AMPLIFICATION AND CLONING OF SF3B3 GENE INTO PMXS-FLAG VECTOR USING RECOMBINATION CLONING KIT

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Abstract: Alternative splicing is a crucial process for regulating gene expression in eukaryotes, and dysregulation of this process has been implicated in multiple diseases, including cancer. SF3B3 is a key splicing factor that has been shown to be upregulated in various cancers, including colon cancer, renal cell carcinoma, hepatocellular carcinoma, and breast cancer. Mutations in SF3B3 have been found to promote the proliferation, colony formation, migration, and invasion of liver cancer cells. In this study, we report the successful construction of an overexpression vector for SF3B3 using homologous recombination. The pMXs-SF3B3 recombinant plasmid serves as a foundation for further investigation into the function of SF3B3 in tumorigenesis. The link between splicing and transcription and the potential of spliceosome components as therapeutic targets for cancer treatment is highlighted by this study.

Keywords: SF3B3, alternative splicing, homologous recombination, colorectal cancer, hepatocellular carcinoma

Introduction

Alternative splicing is a critical process that generates multiple transcripts from a single gene, thereby providing a diverse repertoire of proteins [1]. Aberrant splicing has been implicated in the pathogenesis of various diseases, including cancer. SF3B3 is a crucial component of the spliceosome complex that regulates alternative splicing. SF3B3 has been shown to be upregulated in various cancers, including colon cancer, renal cell carcinoma, hepatocellular carcinoma, and breast cancer. Furthermore, SF3B3 mutants have been found to promote the proliferation, colony formation, migration, and invasion of liver cancer cells [2]. Understanding the function of SF3B3 in tumorigenesis may provide new insights into the mechanisms of cancer development and aid in the identification of new therapeutic targets for cancer treatment. In this study, we constructed a recombinant plasmid for the overexpression of SF3B3 using homologous recombination. The successful construction of the pMXs-SF3B3 plasmid provides a starting point for further research on the function of SF3B3 in tumors. The potential of spliceosome components as therapeutic targets for cancer treatment is also highlighted.

Materials and methods 1. Reagents and cells pMXs-Flag vector (Addgene, Cambridge, MA, USA); human colon cancer cell line SW620

(Shanghai Fuheng Biological Company, Shanghai, China); restriction enzyme Pme I (NEB, USA); and high-fidelity DNA polymerase (Takara Bio.Gold Mix, green); DNA purification and recovery kit (Tiangen, Beijing, China); SoSoo recombinant cloning kit (Beijing Qingke Xinye Biotechnology Co.); DH5α (Biomed); and DNA maker (Biyuntian Biotechnology) were purchased from the indicated manufacturers.

2. Methods

2.1 Primer design and synthesis

To construct the SF3B3 overexpression plasmid, SF3B3 gene was subcloned intothe v ector pMXs-Flag, the sequences was obtained in Genbank (NM_012426.5) and then h omologous recombination primers were designed and synthesized. Theprimers sequence as following: (forward primer) 5'-GTTAATTAAGGATCCGTTTGCCACCATGTTTCTG TACAACTTAACCTTGCAG-3' And (reverse primer) 5'-CGTCCTTGTAGTCTTGTTTC AGAAGGCGTAGCGGGTC-3', Pme I was selectedas the single restriction site, and th e primer sequence was synthesized by KincoBio (Beijing, China).

2.2 Amplification and recovery of SF3B3 cDNA

Total RNA was extracted from colon cancer cell SW620 using Trizol regent (Invitrogen, United States) and the RNA were reverse transcribed into cDNA. Using 1 μL cDNA as template, 22 μL Gold Mix (green), 1 μL SF3B3-HR-FP (10 μmol/L), 1 μL SF3B3-HR-RP (10 μmol/L),total system 25 μL. The PCR reaction program: Pre-denaturation at 98 °C for 2 min, denaturation at 98 °C for 10 s, annealing at 57 °C for 15 s, extension at 72 °C for 1 min, 35 cycles were performed. Lastly, amplified fragments were detected by 1% agarose gel electrophoresis, and target band were recovered.

2.3 Linearzation of pMXs-Flag vector pMXs-Flag vector was digested by restriction endonuclease Pme I. The single enzyme digestion system contain pMXs-Flag plasmid(10 μL), 10 x NEB Buffer (2 μL), ddH2O (7 μL), Pme I (1 μL), the Enzyme digestion condition was 37 ℃ overnight. The digested products were detected using 1% agarose gel electrophoresis and the target fragment were recovered.

2.4 Construction of pMXs-SF3B3 retroviral overexpression vector

To create the pMXs-SF3B3 vector, the SF3B3 fragment was insert into the pMXs-flag plasmid based on the principle of homologous recombination, according to the manufacture of So Soo recombination cloning kit. The system was linearized pMXs-Flag plasmid 1 μL, SF3B3 cDNA fragment 4 μL, 2 x So Soo Mix 5 μL, total volume 10 μL. Water bath at 50 °C for 20 minutes, cooling on ice, the ligation products were transformed into DH-5α competent cells, the transformed product was inoculated on Amp-LB agar plates, and incubated at 37°C for 16 hours. Multiple colonies grow on the agar plate, picked 7 positive clones to prepare bacterial solution, and perform colony PCR to identify the positive SF3B3 gene. The positive clone bacterial liquid were send to Kinco Biotech for sequencing and analysis.

Results

1. **Amplification of SF3B3 gene**

Total RNA from SW620 cells was extracted, and then reverse-transcribed into cDNA. The SF3B3 gene were synthesized by PCR amplification using the specific homology arms primers, and the amplified products were identified by agarose gel electrophoresis. The results are shown in Figure 1. The band size of the PCR product is consistent with the theoretical size of
SF3B3 (3834bp).

2. **Construction and sequence analysis of recombinant plasmid**

The pMXx-Flag was digested with Pme I (Figure 2A), the linearized pMXx-Flag was connected with the purified SF3B3 amplified product (Figure 2B) to construct a pMXx-FlagSF3B3 plasmid. Next, the components of connection were transformed in to DH5α. Seven colonies were randomly selected for colony

PCR identification, and 3 were found to be positive clones (Figure 2C). The bacterial solution N0.7 was selected for sequencing, and it was found that the SF3B3 coding region sequence was recombined into pMXs-Flag (Figure 2D). The correctly sequenced pMXs-SF3B3 recombinant plasmid and pMXs-Flag plasmid were identified by agarose gel electrophoresis, and the sequence length was consistent with the size in theory (Figure 2E).

Discussion

In previous expriments, analysis of TCGA database showed that the expression level of SF3B3 in colon cancer tissues was significantly higher than that in normal tissues, consistent with the results of RT-PCR and Western blotting methods in our study. In human colon cancer cell lines SW620 and SW480 with different metastatic ability, the differential expression of multiple splicing factor mRNA levels was detected, and found that SF3B3 was the most highly expressed in SW620. Studies have found that SF3B3 expression is upregulated in ccRCC, for patients with stage II and III kidney cancer, the high expression of SF3B3 is related to its poor prognosis [15]. SF3B3 is also involved in the regulation of cancer metastasis including breast cancer and hepatocellular cancinoma[16][17]. More studies have shown that SF3B3 mutants can promote the proliferation, colony formation, migration and invasion of liver cancer cells [18]. Splicing factors and their mutants regulate the alternative splicing of multiple genes involved in tumorigenesis. As a therapeutic target, these splicing factors can provide prognostic and predictive effects for a variety of cancers.

Conclusion

In this study, we extracted total RNA from SW620, and obtained the SF3B3 gene sequence by reverse transcription and polymerase chain reaction. Recombination into the pMXs-Flag vector, the successful recombinant pMXs-SF3B3 plasmid were confirmed by colony PCR, sequencing and other methods. The successful contruction of pMXs-SF3B3 recombinant plasmid may provide ideas for the follow-up SF3B3 in-depth study to find new targets for tumor therapy.

Disclosure of interest

The authors report no conflict of interest.

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