CLONING AND DETERMINING OF BAC GENE AND Bcl-2 AND CDK4 EXPRESSION ON ASCITES HEPATOMA CELL LINE Hca-F25/25CL-16A3

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Abstract

Objective: To study the mechanism of cancer, the DNA for BAC was cloned from an ascites hepatoma cell line Hca-F25/CL-16A3 using PCR. Methods: The nucleotide sequences were determined using ABI PRISM™ 377 DNA sequencer. The expression of bcl-2 and CDK4 gene were determined using immunohistochemistry. Results: The sequences of BAC segment on Hca-F25/CL-16A3 have nearly identical sequences with human BAC. The bcl-2 and CDK4 are highly expression on this cell line. Conclusion: The highly expression of bcl-2 and CDK4 may the one of mechanisms for tumor growth.

Key words: BAC gene, bcl-2, CDK4, Hca-F25/CL-16A3.

A human genomic BAC library had been constructed by Shuichi Asakawa et al. using high molecular weight DNA from a pre-pro-B cell line. This BAC library consists of 96,000 clones with an average DNA insert size of 110 kb, covering the human genome approximately 3 times.[1] The division cycle of eukaryotic cells is regulated by a family of protein kinases known as the cyclin-dependent kinases (CDKs). The sequential activation of individual members of this family and their consequent phosphorylation of critical substrates promotes orderly progression through the cell cycle. The complexes formed by CDK4 and the D-type cyclin have been strongly implicated in cell proliferation during the G1 phase.[2] The bcl-2 is the important mediators of apoptosis. Bcl-2, located at chromosome locus 18q, encodes a 26 kilodalton protein which resides in the mitochondrial membrane, endoplasmic reticulum and nuclear envelope. When expressed in tumor it prolongs cell survival and rescues them from apoptosis induced by a variety of agents.[3] In this paper, the sequences of BAC and express of CDK4 and bcl-2 are determined, to study the molecular mechanism of cancer.

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MATERIALS AND METHODS

Cell Line, DNA Extraction, and Expression of bcl-2 and CDK4 Gene

The cell line analyzed was Hca-F25/CL-16A3 (come from mice). DNA was extracted and expression of bcl-2 and CDK4 as described previously.[4]

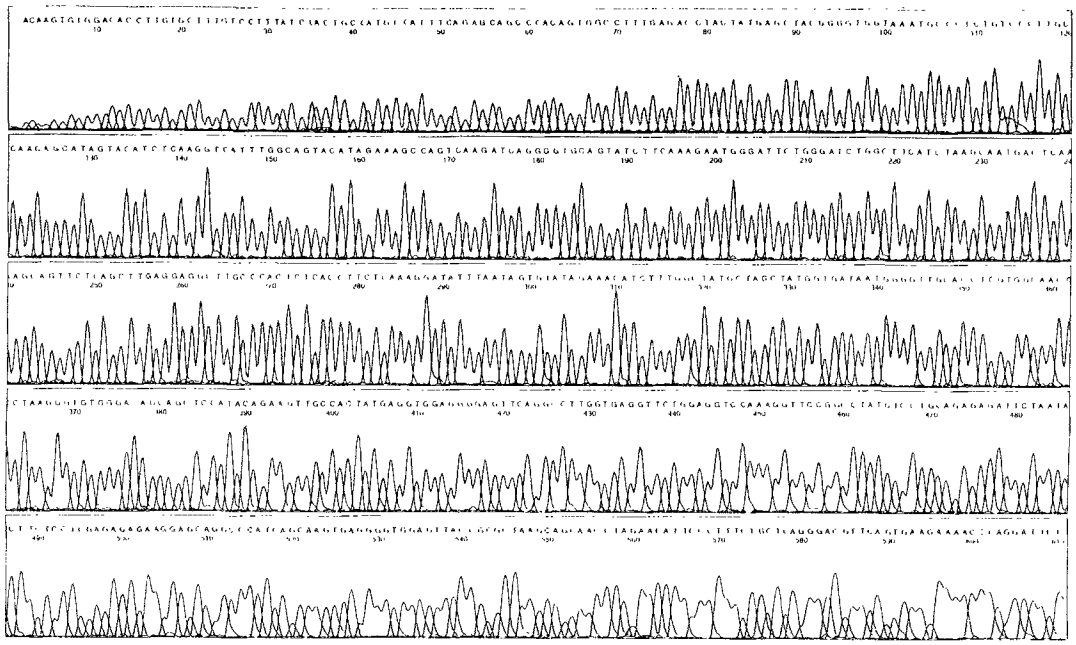
Cloning and Determining Sequences for BAC Gene

Primers 1 for BAC gene were (5'-3'): CTGATCCCGTCCTCCACT. Primers 2 for BAC gene were (5'-3'): ACCATTGCCCAAGTTCAG. PCR condition: amplification of BAC with primers 1 and 2 consisted of 5-min denaturation at 95°C, followed by 1 cycle of 60s at 94°C, 30 cycles of 60s at 98°C, 30 cycles of 30s at 50°C, 30 cycles of 60s at 72°C. PCR product was collected and used for sequencing by the dideoxy chain termination method using ABI PRISM™ 377 DNA Sequencer.

RESULTS AND DISCUSSION

The sequence of BAC gene of Hca-F25/CL-16A3 is showed on Figure 1.

Human BAC clone RG331C24 from 7q21, complete sequence length is 136, 150. The sequence of Hca-F25/CL-16A3 cell line BAC have some identities. The result is showed on Figure 2.



1	ACAAGTGTGG	ACACCTTGTG	CTTTGTCCTT	TATCCACTGC	CATGCCATTT
51	CAGAGCAGCC	CACAGTGGCC	TTTGAGACCT	ACTATGAGCT	ACGGGGTGGT
101	AAATGCCCTC	TGTCCCTTGC	CAACAGCATA	GTACATCTCA	AGGTCATTTG
151	GCAGTACATA	GAAAGCCAGT	CAAGATCAGG	GGTGCAGTAT	CTTCAAAGAA
201	TGGGATTCTG	GGATCTGGCT	TCATCTAAGC	AATGACTCAA	AGCAGTTCTC
251	AGCTTGAGGA	GGCTTGCCCA	CTCTCACCTT	CTCAAAGGAT	ATTTAATAGT
301	GTATAGAAAC	ATCTTTGGCT	ATGCTAGCTA	TGGTGATAAT	GGGGTTGCAC
351	CTCGTGGCAA	CCCTAAGGGT	GTGGGACAGC	AGCTCCATAC	AGAAGTTGCC
401	ACTATGAGGT	GGAGGGGAGT	TCAGGCCTTG	GTGAGGTTCT	GGAGGTCCAA
451	AGGTTCCGGC	CTATGTCCTG	CAGAGAGATT	CTAATACTTC	TCCTCGAGAG
501	AGAAGGAGCA	GGCCCATCAG	CAAGTGAGGG	GTGGAGTTAC	CGCGCTAAGC
551	AGCAACCTAG	AACATTCCCT	TTCTGCTCAG	GGACGTTCAG	TGAAGAAAAC

Fig. 1. The sequence of BAC gene

Hca-F25/CL-16A3 cell line BAC:

461 TATGTCCTGCAGAGAGATTCTAATACTTCTCCTCGA 496

Human BAC:

3846 TATGTCCTGCAGACAAATTCCAATACTTCTTCACTA 3881

Fig. 2. Comparing with identities of BAC gene of human and Hca-F25/CL-16A3

The result suggest that BAC gene exists in Hca-F25/CL-16A3 cell line, the sequence of BAC segment on Hca-F25/CL-16A3 have nearly identical sequence with human BAC, but its function is studied further.

Development of cancer is the result of the accumulation effects of multiple genes, many of which include oncogenes and tumor suppressor genes. However, recently attention has been focused on apoptosis genes. Apoptosis is a common mode of cell loss in tumor, where it occurs both spontaneously and as a result of cytotoxic therapy. In some cancers the rate of spontaneous cell loss even approaches to that of cell production. Consequently, genetic changes which protect cell programmed death may have a significant effect not only on rate of a tumor growth but also on the ability of tumor cell to survive in the hostile environment.[5] Bcl-2 gene is one of the inhibit apoptosis genes, and it highly expression on Hca-F25/CL-16A3 cell line (Figure 3). This result indicate that bcl-2 highly expression is one of the mechanisms for tumor growth.

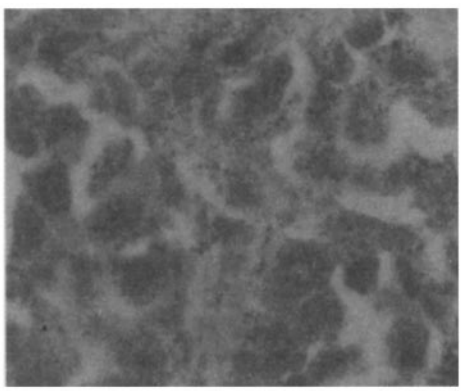


Fig. 3. The expression of bcl-2

The inhibition of CDK4 might be related to the interaction between P16 and CDK4. In some tumor, nearly P16 is not expression and CDK4 is highly expression, this is because it lacks the inhibition of P16. CDK4 is highly expression on this cell line (Figure 4). It may be the reason that the tumor grows.

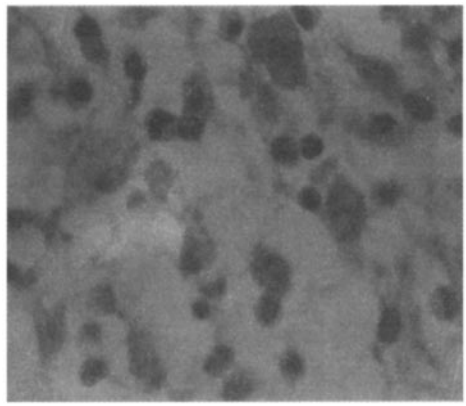


Fig. 4. The expression of CDK4

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