Chinese Journal of Cancer Research 9(2):107 — 110, 1997

THE EXPRESSION OF CDK4 GENE IN HUMAN BREAST CANCER

Xiao Weiqun 肖 卫 群

Department of Radiotherapy, Beijing Institute for Cancer Research, 100034, Beijing

Purpose: To analyze the genetic changes of CDK4 gene and expression of CDK4 mRNA in the breast cancer.

Materials and methods: We obtained a fragment of CDK4 gene, from many kinds of human cancer or normal tissues by PCR .Taking it as a probe, using the methods of Southern and Dot blotting, we analyzed the role of CDK4 gene in the occurrence and development of human breast cancer.

Results: CDK4 gene appeared amplification and its mRNA overexpressed clearly in the breast cell line(MDA231). In human breast cancer tissues, CDK4 mRNA expressed much more than normal breast tissues($p<0.05$).

Conclusion: in the tumorigenesis process, the regulative role of CDK4 is important. its abnormal amplification or expression will cause cell division cycle into a disorder one, then the cell may enter an unlimited proliferation cycle.

Key words: CDK4 gene, amplification, overexpression, tumorigenesis.

More and more experiments suggested that there is very close relationship between cell cycle control and tumorigenesis. How did the normal cells developed into cancer cells is not only the result of that some oncogenes were activated. In order to find the role of CDK4, which is the main cyclinD-dependented kinase in G1 phase, in breast cancer genesis, we used the CDK4 fragment as a probe to analyze by Southern and Dot blotting.

Accepted May 10, 1997

MATERIALS AND METHODS

Cell lines

K562, HL-60, PG, Hela, 2BS (from Dept. of Biology in Beijing Normal University), only the 2BS is a normal cell line(human embryonic lung fibroblast cell line), others are cancer cell lines. KG1 α (from Chinese Institute for Blood Research), MDA231 from (Cancer Hospital, Chinese Academy of Medical Sciences). Normal and cancer tissues of breast are come from The First Hospital of Beijing Medical University.

PCR, Purification and Sequencing

We designed and synthesized a pair of primers CD5-CD3, CD5: 5'T(T/C/A)(C/A/G)TICAICGIGA (T/C)(C/A/G)TNAA 3' CD3: 5'A(C/T/G)ITCIGGIG (A/C/G)ICG (A/G)TACC3'(Synthesized in IMBC Lab. of Singapore), and extracted mRNA from many kinds of human normal and cancer cell lines by the method of *Quickprep™ MicromRNA Purifi-cation Kit.* Some PCR products were obtained by *First Strand cDNA Synthesis Kit and GeneAmp PCR Reagent Kit.* Sequenced by Sanger bideoxidization

Preparing probes and Labeling

The fragments of CDK4 were linked with plasmid puc18 , and transferred into DH5 α . We extracted the plasmid DNA from the positive colonies and purified the CDK4 fragment after restrictive enzyme reaction, then labeled them with α-^{32}p-dCTP by *random Primer-a-Gene Labeling System.*

Southern Blotting

Total DNA were extracted from cells by phenol chloroform method, and 10ug of DNA were digested with EcoR I and Smal I , then were run electrophoresis in 0.7% agarose gels, and transferred to the nitrocellulose filters with 20 × SSC. The filters were prehybridized in a solution containing 50% Formamide, 5 × SSC. 5 × Denhardt's 0.5% SDS , 200 ug/ml denatured salmon DNA for 4h at 42 ℃ , then hybridized with α-^{32}p-dCTP-labeled cDNA probe for 12-16h at 42 ℃ , then washed three times in 2 × SSC/0.1%SDS for 5min at room temperature, twice in 0.1×SSC/ 0.1% SDS for 30 min at 65 ℃, and twice in 0.1 × SSC for 10 min at room temperature. Then the filters were exposed for autoradiography.

Dot Blotting

Total cellular RNA was extracted by one step method. The RNA were electrophoresed on 1.2% agarose gels containing 2.2 M formaldehyde, and transferred to the nitrocellulose filters in 20 × SSC. The RNA blots were hybridized with α-^{32}p-dCTP-labeled probe for 20 hours at 42 ℃, a solution containing 5 × SSC, 50%formamide, 5 × Denhart's reagent, and 0.1%SDS. The filters were washed twice with 0.2 × SSC/ 0.1%SDS at 55 ℃ and at 60 ℃ for 40min, and one with 0.1 × SSC/ 0.1%SDS at 60 ℃ for 40 min , following by autoradiography.

RESULTS

Following the conservative regions of CDKs gene family, designed a pair of primers CD5-CD3, taking them as templates, obtained several different size fragments: 150bp, 290bp, 300bp,580bp (Figure 1). Purified them by low-melt agarose electrophoresis, linked them with plasmid puc18, transferred and amplified, extracted the plasmid DNA from positive colonies, obtained the fragment of 300bp (Figure 2).

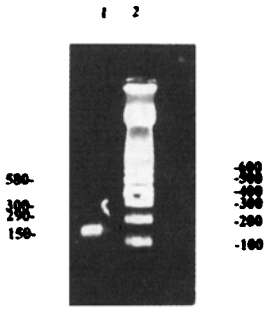

Fig 1 . PCR Products: electrophoresis on 1.5% agarose gel, stained by EB
lane1:PCR products amplified by primer CD5-CD3, size about 150bp,290bp,300bp,580bp
lane2: DNA molecular weight marker (100base-pair)

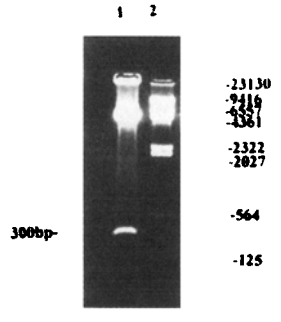

Fig 2. DNA fragment (300bp) obtained from amplified plasmid . digested by EcoR I and Hind III
lane1:300bp DNA fragment and plasmid puc18.
lane2: λ DNA /Hind III molecular weight marker.

Sequencing by Sanger bideoxidization

Compared with mouse published CDK4 gene sequence, the homogeneous degree about 96% (Figure 3).

1 ACGTCAAGGTCACCCTAGCGTTTGAGCATATAGACCAGGACCTGA 45
257 ACATCAAGGTCACCCTAGTGTTTGAGCATATAGACCAGGACCTGA 301

46 GGACATACCTGGACAAAGCACCTCCACCGGGCCTGCCGGTTGAGA 90
302 GGACATACCTGGACAAAGCACCTCCACCGGGCCTGCCGGTTGAGA 346

91 CCGTTAAGGATCTAATGCGTCAGTTTCTAAGCGGCCTGGATTTTC 135
347 CCATTAAGGATCTAATGCGTCAGTTTCTAAGCGGCCTGGATTTTC 391

136 TTCATGCAAACTGCATCGTGCAGCGGGATGTGAAGCCAGAGAACA 180
392 TTCATGCAAACTGCATTGTTCACCGGGACCTGAAGCCAGAGAACA 436

181 TTCTAGTGACAAGTAATGGGACCGTCAAGCTGGCTGACTTTGGCC 225
437 TTCTAGTGACAAGTAATGGGACCGTCAAGCTGGCTGACTTTGGCC 481

226 TAGCTAGAATCTACAGCTACCAGATGGCC 254
482 TAGCTAGAATCTACAGCTACCAGATGGCC 510

Fig 3. The sequence of the 300bp fragment
1-254 is the 300bp DNA fragment , 257-510 is a fragment CDK4(PSK-J3) sequence of mouse

Southern Blotting Analysis and Dot Blotting Analysis of CDK4 Gene

Analyzed the genetic changes of CDK4 DNA and expression of CDK4 mRNA in many kinds of normal and cancer cell lines. The results of Southern blotting indicated that when comparing with normal cell line 2BS, the CDK4 gene was amplified in MDA231 and K562, but not amplified in other cell lines(Figure 4). From the Dot blotting, observed that CDK4 mRNA were overexpressed in the KG1 α and MDA231 cell lines (Figure 5).

Analyzed the expression of mRNA of CDK4 gene in breast cancer and normal tissues. The results suggested that the expression of CDK4 mRNA in cancer tissues are all higher than those of normal tissues(P<0.05).

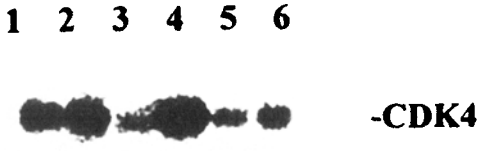

Fig 4. Southern blot analysis of CDK4 gene

The genomic DNAs(10ug) digested with EcoR I and subjected to Southern blotting and hybridized with α- ^{32}p -dCTP-labeled CDK4 cDNA probe
1:2BS, 2: K562, 3: KG1 α , 4:MDA231, 5:Hela, 6:PG

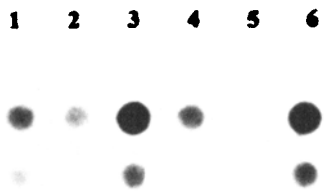

Fig 5. Dot blotting analysis of CDK4 gene expression in cell lines hybridized with α -^{32}p-dCTP-labeled CDK4 cDNA probe
1: K562, 2: HL-60, 3: KG1 α , 4:2BS, 5: Hela, 6: MDA231

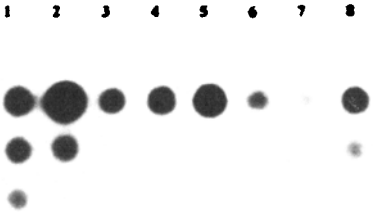

Fig 6. Dot blot analysis of CDK4 gene expression in breast cancer tissue hybridized with α -^{32}p-dCTP-labeled CDK4 cDNA probe, 1,3,6,7 are normal breast tissues, 2,4,5,8 are breast cancer tissues. Among them 1 and 2, 3 and 4, 7 and 8 are come from same patients, 5 and 6come from different normal and cancer patients.

DISCUSSION

More and more experiments suggested that there was very close relationship between cell cycle

control and tumorigenesis.[1-4] With the developing of cell cycle control research, people have more comprehension of cancer or tumor genesis. How did the normal cells developed into cancer cells is not only the result of that some oncogenes were activated or some suppressor genes were lost or mutated. A series of control factors in cell cycle control, such as Rb, P53, CKI, cyclins and CDKs, all can cause tumorigenesis, if they are abnormal. The tumorigenesis is closely related to several checkpoints in cell cycle control, especially to the restrictive checkpoint(R point) in G1 phase.

Starting at the R point in G1 phase is a complex and delicate process. A lot of experiments suggested that activated CDK4/cyclinDs are the main controllors at R point, they are located at a center of message spreading, they phosphate a series of substrates.[5,6] If CDK4 or cyclinDs appear abnormal expression or abnormal amplification in the genomic chromosome, they may cause cells not to enter a divisive cycle normally when added growth factors, and the cell cycle not to stop when there are negative growth factors. Those results may transform normal cells into cancer or tumor cells. So people express more interest in these genes at present.

Breast cancer has been one of the highest frequency of lethal diseases of woman, more and younger woman are subjected to this terrible disease. The abnormality in the cell cycle control may be one of the causes. Naoshi reported cyclinD1 over-expressed and appeared abnormal amplification in breast cancer tissues[7]. K. Keyomarsi et al., also reported cyclin E overexpressed in breast cell lines, and in clinic research, people attempt to use cyclin E as one index in diagnosis to analyze the breast cancer[8]. People were convinced in the clinic analysis, using the assay of cell cycle control is necessary. To find more relationship between these two research fields is a meaning work.

As an main controllor and an important part of cyclin Ds/CDK4 kinase complex, CDK4 may be an important factor in the breast tumorigenesis. In order to find the role of CDK4 gene in breast cancer genesis, we used the CDK4 fragment as a probe to analyze by Southern and Dot blotting. The results indicated that the cDNA of CDK4 gene had been amplified in breast cancer cell line(MDA231) more than normal cell lines, and its mRNA had been overexpressed in MDA231 and in human breast cancer tissue than those normal controls. Those results suggested that in the breast tumorigenesis, as cyclin D and cyclin E,CDK4 is an important factor, its abnormal expression will cause breast cell division control to enter a disorder process. The high lever CDK4 in the cell will cause the G1 phase in breast cells to shorten largely, then will increase the rate of cell proliferation rapidly. The high level of CDK4 may also can cause the supervisory role of the R checkpoint to lose itsefficacy, then cells may proliferate unlimitedly. The abnormal CDK4 gene may be one cause of breast tumorigenesis directly or indirectly. We still need to make much deeply experiments to test it. I think this study, combining the research on cell cycle control with the research on the prevention and treatment of tumor, will be a useful attempt.

REFERENCES

1. Motoaki O. Cyclin-dependent regulation of G1 in mammalian fibroblasts. Science. 1993; 259: 1908.
2. Motokura T, Bloom T, Kim HG, et al. A novel cyclin encoded by a bcl 1-linked candidate oncogene. Nature 1991; 350: 512.
3. Cantley LC, Auger KR, Carpenter C, et al. Oncogenes and signal transduction. Cell 1991; 64: 281.
4. Cohen S, Hiroshi Ushiro, Christa stoscheck, et al. A native 170,000 epidermal growth factor receptor-kinase complex from shed plasma membrane vesicles. J Biol Chem 1982; 257: 1523.
5. Ewen. Phosphorylation at Thr167 is required for S. prombe $P34^{cdc2}$ function. EMBO J 1991; 10: 3297.
6. Matsushine. D-type cyclin-dependent kinase activity in mammalian. Cells Mol Cell Biol 1994; 14: 2066.
7. Naoshi Nishida, Yoshihiro Fukuda, Toshiki Komeda, et al. Amplification and overexpression of the cyclin D1 gene in aggressive human hepatocellular carcinoma. Cancer Res 1994; 54: 3107.
8. Keyomarsi., Nuala O'leary, Gyongyi Molnar, et al. Cyclin E, A potential prognostic marker for breast cancer. Cancer Res 1994; 54: 380.