Isolation, Amplification Approaches of Human Pokemon gene (ZBTB7) and Incorporation into Yeast Vector

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Abstract

The transcription repressor protein ZBTB7 (FBI1, BTB-ZF protein LRF) known as a Pokemon is critical factor in oncogenesis which is encoded by ZBTB7 gene. ZBTB7 processes by controlling the pathways that are required to transform normal cell to cancerous one. It produces its effects by repressing the function of other proteins including a tumor suppressor protein ARF leading to lymphomas and other oncogenic effects and present in very high level in certain type of B cell. So the structural study of the translation product of this gene is an important task to know about the carcinoferous effect of ZBTB7 along with their interactive pattern with co-repressor which prone transcription repression by stopping the activity of transcription factors. In the present project DNA binding domain of ZBtB7 gene's cDNA is developed and cloned into suitable yeast vector.

Keywords: *Isolation, amplification, Pokemon gene (ZBTB7), yeast vector*

Introduction

Many cells normally undergo a programmed form of death (apoptosis). Activated oncogenes can cause those cells to survive and proliferate instead. Most oncogenes require an additional step, such as mutations in another gene, or environmental factors, such as viral infection, to cause cancer [1]. The first oncogene was discovered in 1970 and was termed src (pronounced *sarc* as in *sarcoma*) [2]. Src was in fact first discovered as an oncogene in a chicken retrovirus. Experiments performed by Dr G. Steve Martin of the University of California, Berkeley demonstrated that the SRC was indeed the oncogene of the virus. [3] In 1976 Drs. J. Michael Bishop and Harold E. Varmus of the University of California, San Francisco demonstrated that oncogenes were defective proto-oncogenes, found in many organisms including humans. For this discovery Bishop and Varmus were awarded the Nobel Prize in 1989. Since the 1970s, dozens of oncogenes have been identified in human cancer. Many cancer drugs target those DNA sequences and their products [4]. Most, if not all, cancer cells contain genetic damage that appears to be the responsible event leading to tumorigenesis. The genetic damage present in a parental tumorigenic cell is maintained (i.e. not correctable) such that it is a heritable trait of all cells of subsequent generations. Genetic damage found in cancer cells is of two types:

1. Dominant and the genes have been termed **proto-oncogenes**. The distinction between the terms proto-oncogene and oncogene relates to the activity of the protein product of the gene. A proto-oncogene is a gene whose protein product has the capacity to induce cellular transformation given it sustains some genetic insult. An oncogene is a gene that has sustained some genetic damage and, therefore, produces a protein capable that were originally identified as resident in transforming retroviruses were initially designated as **c–** indicative of the cellular origin as opposed to of cellular transformation.

The process of activation of proto-oncogenes to oncogenes can include retroviral transduction or retroviral integration (see below), point mutations, insertion mutations, gene amplification, chromosomal translocation and/or protein-protein interactions.

Proto-oncogenes can be classified into many different groups based upon their normal function within cells or based upon sequence homology to other known proteins. As predicted, proto-oncogenes have been identified at all levels of the various signal transduction

cascades that control cell growth, proliferation and differentiation. The list of proto-oncogenes identified to date is too lengthy to include here, therefore, only those genes that have been highly characterized are described. Proto-oncogenes **v–** to signify original identification in retroviruses.

2. Recessive and the genes variously termed tumor suppressors, growth suppressors, recessive oncogenes or anti-oncogenes.

Given the complexity of inducing and regulating cellular growth, proliferation and differentiation, it was suspected for many years that genetic damage to genes encoding growth factors, growth factor receptors and/or the proteins of the various signal transduction cascades would lead to cellular transformation [5]. This suspicion has proven true with the identification of numerous genes, whose products function in cellular signaling, that are involved in some way in the genesis of the tumorigenic state. The majority of these proto-oncogenes were identified by either of two means: as the transforming genes (oncogenes) of transforming retroviruses or through transfection of DNA from tumor cell lines into non-transformed cell lines and screening for resultant tumorigenesis.

Pokemon Linked to Aggressive Tumors

The investigators have confirmed Pokémon's cancer-causing role by inserting the oncogene into mice. Pokemon does its damage by repressing the function of other proteins, including a tumor suppressor called "ARF."

The mice used developed aggressive, fatal forms of lymphoma. In further work, using a technique called "tissue micro arrays" to study tumor samples from people with many types of cancer, the researchers have confirmed that Pokemon is present in very high levels in certain types of B-cell and T-cell lymphomas. They also found that tumors with high levels of Pokemon protein were much more likely to be aggressive. [6]

"Pokemon is a member of a family of proteins that are known to be transcription factors and are mutated in human cancer," according Takahiro Maeda, MD, PhD, a postdoctoral research fellow in Dr. Pandolfi's laboratory who was the paper's first author. "It is likely that the protein plays a role in solid tumors as well, and we now have means to specifically interfere with the activity of these transcription factors."

Gene Amplification

An example of gene amplification, which usually occurs during tumor progression, is the amplification of the dihydrofolate reductase gene (*DHFR*) in methotrexate-resistant acute lymphoblastic leukemia. Amplification of *DHFR* is accompanied by cytogenetic alterations that mirror amplification of oncogenes. The amplified DNA segment usually involves several hundred kilobases and can contain many genes. Members of four different oncogene families are often amplified: MYC, cyclin D1 (or CCND1), EGFR, and RAS [7]. *MYC* is amplified in small-cell lung cancer, breast cancer, esophageal cancer, cervical cancer, ovarian cancer, and head and neck cancer, whereas amplification of *NMYC* correlates with an advanced tumor stage.The t(11;14) translocation juxtaposes *CCND1* and immunoglobulin enhancer elements and is characteristic of mantle-cell lymphoma *CCND1* amplification also occurs in breast, esophageal, hepatocellular, and head and neck cancer. *EGFR* (*ERBB1*) is amplified in glioblastoma and head and neck cancer. Amplification of *ERBB2* (also called *HER2/neu*) in breast cancer correlates with a poor prognosis.51 A monoclonal antibody against the product of this oncogene (trastuzumab) is effective in breast cancers that overexpress HER2/neu.

Chromosomal Rearrangements

Recurring chromosomal rearrangements are often detected in hematologic malignancies as well as in some solid tumors.These rearrangements consist mainly of chromosomal translocations and, less frequently, chromosomal inversions. Chromosomal rearrangements can lead to hematologic malignancy via two different mechanisms:

(1) the transcriptional activation of protooncogenes or

(2) the creation of fusion genes.

Gene Activation

The t(8;14)(q24;q32) translocation, found in about 85% of cases of

Burkitt lymphoma, is a well-characterized example of the transcriptional activation of a protooncogene. This chromosomal rearrangement places the c-*myc* gene, located at chromosome band 8q24, under control of regulatory elements from the immunoglobulin heavy chain locus located at 14q32.98 The resulting transcriptional activation of c-*myc*, which encodes a nuclear protein involved in the regulation of cell proliferation, plays a critical [8] role in the development of Burkitt lymphoma.The c-*myc* gene is also activated in some cases of Burkitt lymphoma by translocations involving immunoglobulin light-chain genes.These are t(2;8)(p12;q24), involving the κ locus located at 2p12, and t(8;22)(q24;q11), involving the κ locus at 22q11 .

Although the position of the chromosomal breakpoints relative to the c-*myc* gene may vary considerably in individual cases of Burkitt lymphoma,

Gene Fusion

The first example of gene fusion was discovered through the cloning of the breakpoint of the Philadelphia chromosome in chronic myelogenous leukemia (CML). The t(9;22)(q34;q11) translocation in CML fuses the

c-*abl* gene, normally located at 9q34, with the *bcr* gene at 22q11 (Figure 6-7).The *bcr/abl* fusion, created on the der(22) chromosome, encodes a chimeric protein of 210 kDa, with increased tyrosine kinase activity and abnormal cellular localization.The precise mechanism by which the *bcr/abl* fusion protein contributes to the expansion of the neoplastic myeloid clone is not yet known. The t(9;22) translocation is also found in

up to 20% of cases of acute lymphoblastic leukemia (ALL). In these cases, the breakpoint in the *bcr* gene differs somewhat from that found in

CML, resulting in a 185 kDa bcr/abl fusion protein. It is unclear at this time why the slightly smaller bcr/abl fusion protein leads to such a large difference in neoplastic phenotype. In addition to c-*abl*, two other genes encoding tyrosine kinases are involved in distinct gene fusion events in hematologic malignancy. The various partners in *ALL1* fusions encode a diverse group of proteins, some of which appear to be nuclear proteins with DNA-binding motifs. The ALL1 fusion protein consists of the aminoterminus of ALL1 and the carboxyl terminus of one of a variety of fusion partners. It appears that the critical feature in all ALL1 fusions, including self-fusion, is the uncoupling of the ALL1 amino-terminal domains from the remainder of the ALL1 protein. Solid tumors, especially sarcomas, sometimes have consistent chromosomal translocations that correlate with specific histologic types of tumors. In general, translocations in solid tumors result in gene fusions that encode chimeric oncoproteins. Studies thus far indicate that in sarcomas, the majority of genes fused by translocations encode transcription factors.In myxoid liposarcomas, the t(12;16)(q13;p11) fuses the *FUS* (*TLS*) gene at 16p11 with the *CHOP* gene at 12q13. The FUS protein contains a transactivation domain that is contributed to the FUS/CHOP fusion protein. The CHOP protein, which is a dominant inhibitor of transcription, contributes a protein-binding domain and a presumptive DNA-binding domain to the fusion. Despite knowledge of these structural features, the mechanism of action of the FUS/CHOP oncoproteins is not yet known.

Oncogenes in Cancer Initiation and Progression

When chronic myelogenous leukemia converts to acute leukemia, the malignant clone acquires an additional t(9;22) translocation, an isochromosome , or trisomy of chromosome 8. When follicular lymphoma becomes aggressive, the lymphoma cells often bear a t(8;14) translocation in addition to the original t(14;18) translocation. These findings support the hypothesis that most hematopoietic tumors and soft-tissue sarcomas are initiated by the activation of an oncogene, followed by alterations in tumor-suppressor genes and other oncogenes. In contrast, most carcinomas are initiated by the loss of function of a tumor-suppressor gene, followed by alterations in oncogenes and additional tumor-suppressor genes.This multistep process in human cancer has also been found in mouse models carrying activated oncogenes or inactivated tumor-suppressor genes, in which the duration and aggressiveness of the disease can be changed by introducing into the mouse genome the same sequential genetic alterations observed in human tumors [9-11]. Methylation of CpG islands located in the promoter regions of a number of tumor-suppressor genes has also been considered an important epigenetic step in the process of carcinogenesis. This topic will be covered later in this series.

Oncogenes as Therapeutic Targets

Oncogenic proteins in cancer cells can be targeted by small molecules and, when the oncogenic protein is expressed on the cell surface, by monoclonal antibodies. Table 1 contains a summary of the targets and drugs (small molecules and monoclonal antibodies) being used in the treatment of a variety of human cancers.

Table 1. Cancer Therapies That Target Oncogenic Proteins.

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Anticancer Drug	Target	Disease
Monoclonal antibodies		
Trastuzumab (Herceptin, Genentech)	ERBB2	Breast cancer
Cetuximab (Erbitux, ImClone)	EGFR	Colorectal cancer
Bevacizumab (Avastin, Genentech)	VEGF	Colorectal cancer, non–small-cell lung cancer
Small molecules		
Imatinib (Gleevec, Novartis)	ABL, PDGFR, KIT	Chronic myelogenous leukemia, gastrointestinal stromal tumors, chordoma
Gefitinib (Iressa, AstraZeneca)	EGFR	Non–small-cell lung cancer
Erlotinib (Tarceva, Genentech)	EGFR	Non–small-cell lung cancer
Sorafenib (Nexavar, Bayer/Onyx)	VEGFR, PDGFR, FLT3	Renal-cell carcinoma
Sunitinib (Sutent, Pfizer)	VEGFR, PDGFR, FLT3	Gastrointestinal stromal tumors, renal-cell carcinoma

* EGFR denotes epidermal growth factor receptor, FLT3 FMS-like tyrosine kinase 3, PDGFR platelet-derived growth factor receptor, and VEGF vascular endothelial growth factor.

Imatinib targets the initial step of the multistep process in chronic myelogenous leukemia.The same drug can affect the KIT and PDGFR receptor kinases. Of particular interest are inhibitors of the BCL2 family, which can induce the apoptotic death of cancer cells. In acute promyelocytic leukemia, which is initiated by a t(15;17) chromosome translocation that fuses the *PML* gene to *RARα* (a nuclear receptor for retinoic acids; see Table 2 in the Supplementary Appendix), retinoic acid can induce terminal differentiation and death of APL cells. This modality is called differentiation therapy.

Materials and Method

DNA Preparation from Frozen Tissue

Reagents

Chloroform

EDTA, 0.5 M

Ethanol, absolute

Propanol

Phosphate Buffered Saline (PBS), 1X

Sodium dodecyl sulfate (SDS) solution, 10%

Preparation

DNA buffer (Tris-EDTA)

1 M Tris pH 8.0 20 ml

0.5 M EDTA 20 ml

Sterile water 100 ml

Procedure

1. Put 5 mg of tissue in a petri dish with buffer and divide the tissue into pieces.

2. Take them in centrifuge tubes and keep it in 4°C

for 5 mins

3. Centrifuge for 2 min at 1500 rpm.

4. Remove the supernatant, and wash twice with 1 ml 1X PBS

5. (It is possible to store the pellet at -80°C; in that case, add 1 ml 1X PBS and resuspend the pellet)

6. Remove supernatant and resuspend the pellet in 2.06 ml DNA-buffer.

7. 25 µl 10% SDS, shake gently, and incubate overnight at 35°C temperature

8. If there are still some tissue pieces visible, add 20 µl of 10% SDS and incubate for another 5 hr at 45°C.

9. Add 2.4 ml of Propanol, shake by hand for 5-10 min, and centrifuge at 3000 rpm for 5 min.

10. Pipette the supernatant into a new tube, add 1.2 ml Propanol , and 1.2 ml chloroform shake by hand for 5-10 min, and centrifuge at 3000 rpm for 5 min

11. Pipette the supernatant into a new tube, add 2.4 ml chloroform shake by hand for 5-10 min, and centrifuge at 3000 rpm for 5 min

12. Pipette the supernatant into a new tube, add 25 µl 3 M sodium acetate (pH 5.2) and 5 ml ethanol, shake gently centrifuge it for DNA precipitates.

13. Wash the DNA in 70% ethanol and dry it .

14. Dissolve the DNA in 0.5-1 ml sterile water overnight at 4°C.

RESTRICTION DIGESTION OF YEAST VECTOR

· To the restriction enzyme containing vial, 25 µl of yeast plasmid is added.

· To the above tube, 25µl of 2% assay buffer is added.

· The contents are mixed by tapping the vial for few seconds or briefly spinning at low speed in a micro-centrifuge.

· The vial was incubated in a 37˚C water bath for 1 hour. After 1 hour the vial is removed from water bath and 10µl of gel loading dye is added into the vial and kept on ice.

· 10µl of λDNA from the vial containing only λDNA substrate is transferred into a fresh 1.5 ml appendorff tube.

· Now the sample in all the appendorff tubes is loaded in different wells of 1% agarose gel. When sample loading is over start the electrophoresis set up.

DNA ELECTROPHORESIS

· Measure 1 g Agarose powder and take it in a 500 ml flask.

· Add 100 ml TAE Buffer to the flask.

· Melt the agarose in a microwave or hot water bath until the solution becomes clear. (if using a microwave, heat the solution for several short intervals - only until the solution starts to boil).

· Let the solution cool to about 50-55°C, swirling the flask occasionally so it cools evenly.

· Place the combs in the gel casting tray.

· Pour the melted agarose solution into the casting tray and let cool until it is solid (it should appear milky white).

· Carefully pull out the combs.

· Place the gel in the electrophoresis chamber.

· Add enough TAE Buffer so that there is about 2-3 mm of buffer over the gel.

Loading the gel

· Add 10 µl of each sample to 2 µl of 6X Sample Loading Buffer (make sure the order in which each sample is loaded be recorded).

· Carefully pipette the mixture into separate wells in the gel.

· Pipette 5 µl of the DNA ladder standard into another well of the gel.

Running the gel

· Place the lid on the gel box, connecting the

electrodes.

· Connect the electrode wires to the power supply, making sure the positive (red) and negative (black) are correctly connected.

· Turn on the power supply and maintain around 100 volts.

· Check to make sure the current is running through the buffer by looking for bubble formation on each electrode.

· Check to make sure that the current is running in the correct direction by observing the movement of the blue loading dye – this will take a couple of minutes (it will run in the same direction as the DNA).

· Let the power run until the blue dye approaches the end of the gel.

· Turn off the power.

· Disconnect the wires from the power supply.

· Remove the lid of the electrophoresis chamber.

· Carefully remove the gel.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Ministry of education and all experiments were carried out in accordance with approved guidelines.

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