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Gene Science in Medicine

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The use of gene technology in medicine is shortly reviewed. The diagnosis of hereditary diseases and application to medico-legal science by manipulation of human genes are explained. Its unristricted use may, however, elicit social dilemmas. Gene therapy which is now urgently studied is described more in detail. Ethical problems about gene science in medicine are discussed.

(Gene / DNA diagnosis / Personal identification / Oncogene / Genetherapy / Ethics)

I GENERAL SURVEY OF GENE SCIENCE IN MEDICINE

An evil concept was overwhelming in past decades that the nucleic acid has no value in the practical medicine. Neither has it enzymic activity, nor renders any useful parameter in clinical examinations. Going back to the discovery of the nucleic acid, isolation of DNA from salmon sperm and from human pus implied that : it is an inert material. In this short review I shall argue that the use of nucleic acid is going to be a very useful or indispensable object in the clinical and social medicine in terms of practice.

Good knowledge of DNA studies has been accumulated as to the manipulation of human genes. Various cloned genes such as insulin gene and growth hormone gene were introduced into suitable hosts to produce active materials in larger quantities. Also the engineered gene can lead to the creation of new useful organisms. Technology adopted in the medical science is shared by other biological sciences. The subjects which concern with the provision of new medicines by means of gene engineering are excluded from this review. Only the use of human gene in the clinical and forensic medicine will be discussed.

The rapid and vast expansion of knowledges of the manipulation of genes may elicit chaos unless it is harmonized with its social mileus. The abuse of growth hormone in a human population, for example, may give rise to a new human population with altered body size. Here the social science has a voice. However, social science does not offer eternal standards. Any living organism has never been an object of patented right. This concept premised that the creation of new organism is in the hand of Lord and no concession of life coerced by an individual should be admitted. Gene science can now create new organisms and that the rigid concept has to be amended. More critical and complicated arguments should be inspired on the monopoly of proprietorship and the exclusive use of human gene, such as a case of minisatellite gene treated in the third section.

Stronger arguments should be made when human genes are used to treat pateints suffering from genetic diseases. We remember a case of a gene therapy, which was attempted several years ago and ended with an unhappy story [1]. The gene

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therapy has thus been under the impression among medical doctors that it is a purely theoretical, or worse than that, fancy treatment. Knowledges have been accumulated since then, especially on the gene transfer mediated by the transplantation of bone marrow cells, which have been transfected with human gene carried by a retrovirus vector. We are now confronting, willingly or unwillingly, the actual application of gene therapy. We have to strive to settle ethical problems which inevitably follow.

II DNA DIAGNOSIS

DNA diagnosis of sickle cell anemia is a good example. Changes of amino acid sequence from Pro GluGlu in normal globin to ProValGlu in globin of sickle cell anemia as well as altered base sequence of its gene mutated was identified. The codon 5,6,7 CCTGAGGAG was changed to CCTGTGGAG in the patients. If there exists a restriction enzyme which recognizes the base sequence at mutated site in sickle cell anemia, the restriction fragment of DNA obtained from the patient should give different fragments from those of normal persons. Such restriction enzyme does exist. Mn I was first proposed by Nienhuis [2], but fragments obtained by the digestion with Mn I were two small and inconvenient to be detected by electrophoresis. The next enzyme used was Dde I, which recognizes CTNAG. Geever's group and Kan's group were capable of demonstrating the difference between Dde I fragments of DNA from a patient of sickle cell anemia and from those of a normal person. The fragments obtained from normal person gave two bands whose sizes were 0.17 and 0.20 kb, while a new single band appeared in patients with sickle cell anima instead of these two bands. This new band had a size of 0.37 kb, which was made by the fusion of the two bands due to the absence of Dde I site in the patients. These bands were, however, still too small to be conveniently detected.

Kan's group was able to detect this abnormal band on amnion cells to perform the prenatal diagnosis. However a larger amount of DNA was required for this analysis. Later a new restriction enzyme Mst II which recognizes seven base sequence was introduced by Kan's group [3] and Orkin's group [4]. It gave a large advantage over the use of Dde I. Mst II gave 1.15 kb and 0.2 kb fragment in normal persons, while it gave 1.35 kb in patients (Fig 1 from [4]) (recongnition of seven base apirs theoretically gives rise to a larger restriction fragment than that given by a five-base-recognition enzyme). It enabled to perform the diagnosis by use of a small amount of DNA, eliminating the needs for culturing amnion cells, previously required to obtain a sufficient amount of DNA. Patients are no longer required to have a long waiting time to be informed of the results of the examination. Such differences in patterns of restriction fragments are called RFLP (restriction fragment length polymorphism). Increasing reports are now piled up on RFLP and they give large contribution to the diagnosis of genetic diseases.

Hemophilia can also be diagnosed by the use of an enzyme Bcl, which detects 1.165 kb fragment in stead of 879 bp fragment of normal individual [5] (Fig. 2 from [6]) The mutated site is located in a intron downstream from 18th exon. The mutated site is tightly linked to the affected gene. For the correct diagnosis, rod score should be used for the linkage and the pedigree analysis is needed to assign RFLP to a genetic disease. These arguments are not reviewed here.

III MEDICOLEGAL APPLICATION

DNA is characteristic to each indiviual. It leads to the concept that RPLP can be applied to the identification of individuals if a suitable probe can be used. A probe explained in the second section for the detection of sickle cell anemia recognizes only one change of restriction fragment. Multiple

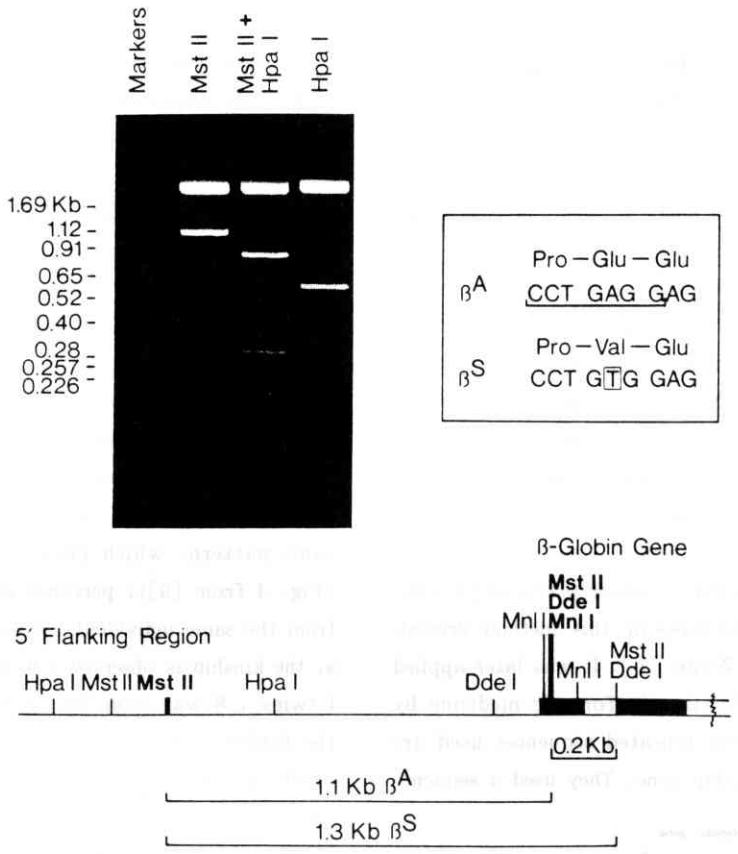

Fig. 1 Restriction fragment of β-globin gene

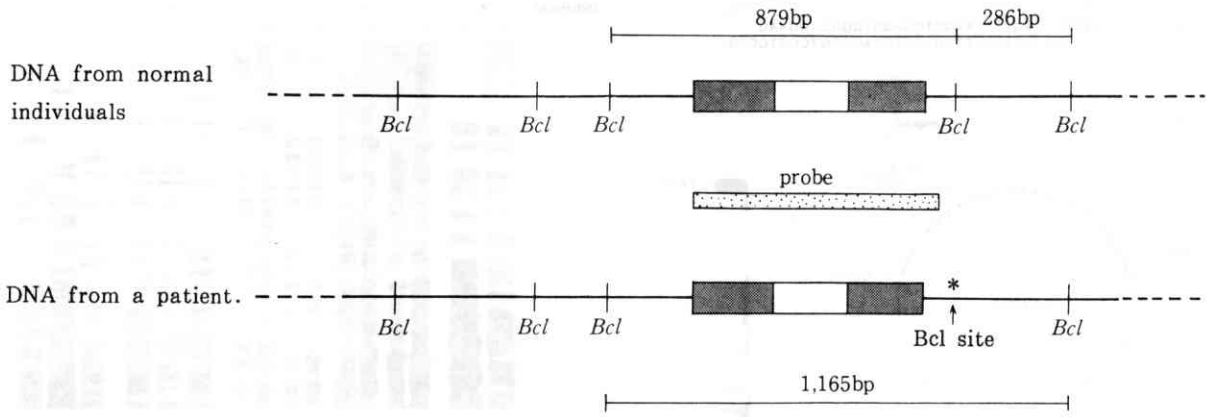

Fig. 2 Detection of Hemophilia gene

differences of restriction fragments should be detected for the personal identification. Accordingly, the probing should be run many times. A special probe was found which takes advantage of the repeated sequence and detects various changes at multiple sites by a single probing. Sequences are repeated during the evolution of mammals and thus the repeated sequences are scattered in the whole genome. The repeats are not always homologous in their sequences as well as in their lengths. Therefore human DNA gives a specific pattern of restriction fragments after digestion with one restriction enzyme and subsequent hybridization with this probe. Such a pattern is characteristic to each individual, in other words, a fingerprint of each individual. It is similar to finger prints of protein. A report making the basis of this method presented by Wyman and White [7]. It was later applied to personal identification in forensic medicine by Jeffrey's group. The repeated sequences used are located near myoglobin gene. They used a sequence present in the second intron of myoglobin gene [8]. This sequence consisted of 4 times repeated 33 bp long sequences, which was flanked by 9 bp at both sides. The repeat of 33 was then excised, ligated tandem and cloned into a vector pUC 13 making pAV 33.7 (Fig. 3 from [8]); The 33 bp sequence was used as a probe to screen human genomic library. Eight clones obtained were designated as 33.1 etc. Each insert varied between 0.2 kb and 2.0 kb in its whole lengths as well as in its unit sequence varing from 16 to 64 bp. One of these recombinants was used as a probe. 1) DNA from blood (B), sperm (S) and transformed lymph oblastoid cells (L) of the same individual show the same pattern, which proves the identy of DNA (Fig. 4 from [9]); personal identification) 4 was from the same individual ; 5,6,7 were from relatives, the kinship is observed ; 9,10 were from identical twins ; 8 was from the mother ; 11 was from the father ; 12,13 were from unrelated persons ; probe a, 33.6 ; probe b, 33.15) 2) DNA from twins were fully identical. (twin diagnosis) 3) Furthermore affer the bands of the twins were substracted from those of mother, the rests coincided clearly to those of father. These bands were

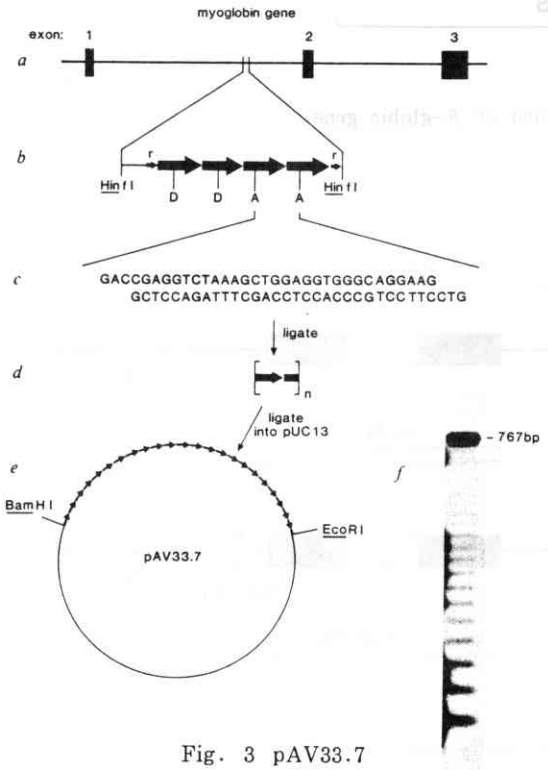

Fig. 3 pAV33.7

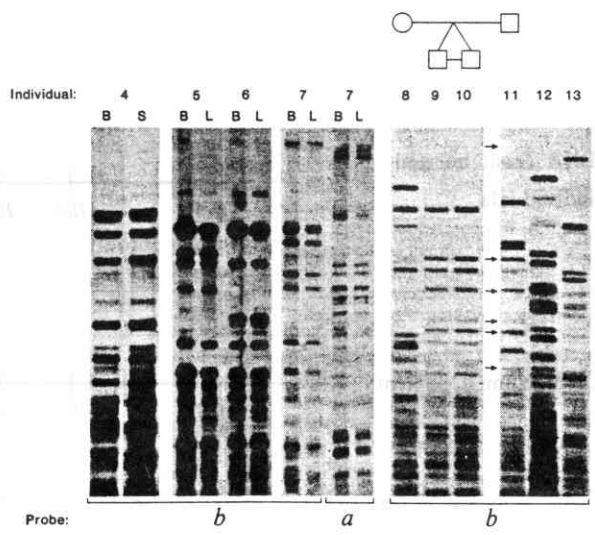

Fig. 4 Personal identification

unrelated the different individual. These patterns given by restriction enzyme fragments makes the paternity confirmation possible. This mothod is now going to be a very powerful tool in the forensic medicine. Its use should be carefully subjected to a human right.

Ⅳ ONCOGENES

The practical and effective contribution of the gene science can not yet be claimed to have its concrete basis in the anticancer struggle. It can not remain in the fringe of medical science and a very short comment should be made here.

Mammalian genes are assembled into a complete system and render a cell to be in a balanced regulation. Once a mutatim occurrs and the cell becomes immortile, immortality is trasmitted within a lineage of the cell, which identifies itself to be a new genetic determinant. In many instances the deterimant is shown to be related to oncogenes. There is no doubt that the characterizations of oncogenes play a great role on the malignancy of tumors. The amplification of oncogenes in various tumors is worth mentioning. It is not directly asociated with the malignancies and not all the cancer have the amplification of oncogenes, the corelation of the malignancy with the amplification of oncogenes was proposed in several kind of tumors. The corvrelation may be of value in prognosis.

I should be stressed that the nature of malignancy is recessive in many cases. The fusion of tumor cells with normal cells gave phenotipically normal cells [10]. The phenotypic expression of recessesive gave requires explanation Tumorigenesis of retinoblastoma was proposed to be induced by homozygosis of a recessive gene [11]. A retinoblastoma gene was cloned and inferred to code for a DNA binding motein [12].

Ⅴ GENE THERAPY

One can easily imagine that the introduction of normal β globin gene into a patient suffering from a sickle cell anemia can cure sickle cell anemia. In such a trial, however, problem is predicted that the increase of normal β-globin produced in the gene-treafed patient might become out of equilibrium to the normal level of α-globin produced in the patient. The balanced regulation of the synthesis of both globins makes the gene therapy of hemoglo binopathia more complicated. A genetic disease in which the failure of a single gene occurs is more easily accessible and is a better candidate for the breakthrough.

The introduction of a new gene into a mammal can be carried out conveniently by transplantation of bone marrow. Though the human gene therapy has not yet been performed at least in its proper application, it seems at present hopeful. Attempts are now being made to introduce a human HPRT gene into mouse bone marrow cells, which were then transplanted to a mouse. Patients suffering from Lesch Nyhan have deficiency of HPRT gene and are under consideration for gene therapy. An example of the transfer of HPRT gene in mice explained [13].

Molony virus was used as a vector. Its use takes advantage of ψ cells. ψ cell harvors a defective virus in its chromosome, which is devoid of a region coding for the packaging of viral RNA into its envelope. ψ cell is itself unable to produce virus, but capable of producing a new virus only when ψ cell is transfected with a suitable recombinant DNA. The recombinant DNA used as a mediator of the human HPRT gene consists of LTR, packaging gene, HPRT gene and antibotic gene (Fig. 5) from [13]. The last gene was used to demonstrate the transfection of cells, namely the appearance of neomycin resistance in foci formed by the transfection. Molony virus has various traits, ecotropic, xenotropic, amphotropic virus.

Amphotropic Molony virus has wide host range and the virus produced from ψam cells are capable of infeting cells from foreign species including human cells.

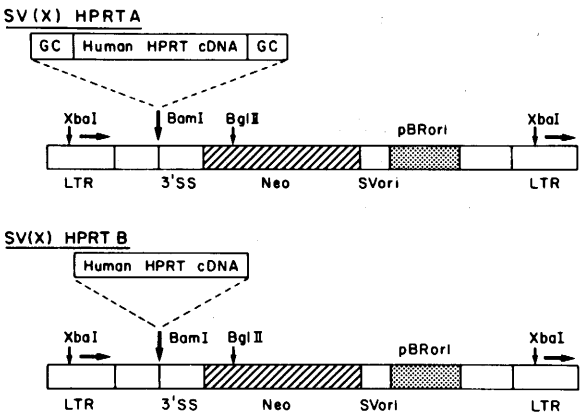

Fig. 5 Cloning of HPTR gene

ψ cells transfected with human HPRT gene which is colinked with neo^r gene gave clones in which both markers cosegregated. It should be mentioned that the prouced virus into which the human gene was cloned must have high titer. In order to achieve the high titer virus, pingpong shuttle infection between ψ2 and ψ am cells was adopted. Virus with high titer could transfect mouse bone marrow cells. Transcript of human HPRT gene was detected in the transfected cells in addition to that of inherent HPRT gene of mouse. Finally it was successfully demonstrated that mouse bone marrow cells, transfected with such artificial virus in vivo, gave product of human HPRT gene demonstrated by use of isoielectric focuussing.

Success of gene therapy in animal experiment encouraged us. With regard to the careful indications how the gene therapy should be ionducted, a commonly acceptable guide line should be established [14]. Firstly, the disease is de facto serious and there is no other way to treat it. The gene transfer of human HPRT gene can be considered, because its severe defect is observed in Lesh Nyhan disease. This disease becomes now one of the first candidate of the gene therapy. Secondly, the excess expression of the newly introduced gene should not be too harmful. Suppose the catastroph which will be elicited by the overproduction of insulin produced by the administer ation of engineered insulin gene. With respect to this point, we must have sufficint knowledge how to control the expression of genes introduced in mammalis by recombinant DNA.

Another problem which is not only of clinical but also of theoretical interest is the tissue specific expression of gene. It is of great interest that the human inslulin gene while was introduced into transgenic mice (introduction of a gene into a germ cell line, which leads to the formation of a adult mouse incorporated with the gene) exerted its function only in the pancreas of the mouse.

VI ETHICAL DILEMMAS

In practicizing DNA test, the advantage of chorionic villus sampling over the amniocentasis is worth discussing. The former is regarded as more acceptable to have abortion of embryo than aborting fetus. The chorionic villus sampling can afford the amount of DNA sufficient to perform the test in a shorter period.

The serious problem is also elicited in performing DNA test. In case a person is at risk with Huntington disease, he will feel doomed to know that the disease will be developed later. His life will be changed, so commented Dr. Rimoin at University of California. There will be a danger of suicide or losing his job. Substance abuse may ensue. Legal question arises ; who decides the DNA test should be done. Should a spouse, should an employer force him to do that? Doctors are also envolved with the problem wether he should tell that to the relatives of the affected person.

If the person at risk does not want to know

that he does carry the gene and does not want to take responsibility of having affected children, should the prenatal diagnosis be still carried out? Some foreign survey showed that about half of the patients at risk with Huntington disease would want to be tested DNA diagnosis.

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