Recent Progress in Ribozyme Antisense Gene Therapy for Autosomal Dominant Retinitis Pigmentosa

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Abstract Retinitis pigmentosa(RP)is a common hereditary retinal dystrophy disease, its pathogenesy is complicated, for the past few years it has become the investigative hot spot. This paper gives an overview on recent advances in ribozyme antisense gene therapy for autosomal dominant retinitis pigmentosa.

Key words Autosomal dominant retinitis pigmentosa; Antisense technology; Ribozyme; Adeno-associated virus(AAV); Stable target site

Retinitis pigmentosa is a hereditary retinal dystrophy disease which is a series of progressing necrosis of retina photoreceptor cells and pigment epithelium and which is nocturnal caecitas, progressing disadvantage campus visualis, hyperpigmentation in eye ground and malo-electroretinogram as clinical feature. With the development of disease retinal arteria and retinal vein are taper and supervene caligo lentis and cataracta glauca atanaphase. Complaint happens at teenager which leads to ablepsia totalis. RP is a most frequent heritage retinal disease which leads to disadvantage optesthesia and blind. Diseaseincidence is from 1/3500 to 1/4000 in the world[1], it involves about 1~2/5000 neonates in the westen countries. It estimates that 1~50 thousand man are troubled by this illness. In our country the ratio of detection gradually upgrades the pathogenesy is complicated which are shot of safe and active therapeutic measure. It is a focal point in department of ophthalmology. This disease is a genetic disease; there are autosomal recessive, autosomal dominant inheritance and X-linked hereditary. There is a handful of patient who appears to double gene mutation[1] or mitochondrial inheritance[2]. Autosomal dominant retinitis pigmentosa has 15%~25%[3] in the RP and has 15 sites. There has been cloned 14 genes[4], The autosomal dominant inheritances pigmentary degeneration of retina family constellation fairly large, the disease incidence is higher than X-linked hereditary, it is an investigative hot spot in recent years.

This articles is an overview about recent progress in ribozyme antisense gene therapy for autosomal dominant retinitis pigmentosa.

The medicative status of autosomal dominant pigmentosa retinae

The main approach therapy means are drug treatment, amphiblestrodes transplantation, and election array gene therapy.

Animal experiment indicate vitamin A that produces a marked effect to ADRP by increasing erythropsins active [5]. Ca_2+ antagon, dilthiazem, is administered by abdominal membrane, which may effect to rd mouse degenerative photoreceptor cells and may conserve visual function[6]. But the pharmacal therapeutic dose may possibly highly exceed dosage that is stipulated by laws at present. We should overview whether dys-results happen after long term application.

There are some patients who improve acuity of vision and campus visualis at some extent after omentum transplantation [7], but the bulk of RP patients is no amelioration after and there are reject reaction[8], postoperative infection and complications. The long-term should be more investigated.

Electron array is used by some Americans but the result is disappointed. And the developing country patients can not afford the expensive price.

ADRP is a common hereditary retinal dystrophy disease with different phaenotypes producing by gene defects. For the disadvantage of therapeusis the gene therapy will became the hot spot in the future.

The investigative advancement of autosomal dominant degeneration gene therapy

ARRP is generated by gene mutation, the default of gene product. With regard to this category normo-

gene is introduced to photoreceptor cells, generates normal gene expression and stabilizing express. The therapy may utilitize. So the supplement unit may be suitable to ARRP[9].

But the reason of ADRP is anomal-gene product accretion which result to photoreceptor cells degeneration. So normal gene can not chalk the goals of treatment. Anomal-gene is deleted or blocked its express by antisense technology. Antisense technology is gene inactivation which utilizes antisense oligonucleotides and/or ribozyme to specically block the mutant gene express. At present we more concentrate on ribozyme. The discoverying of ribozyme hew out a new epoch, as a new gene therapeutic tool it has developed quickly in recent years. It has a dependable prospect in ADRP therapy. Ribozyme is a medium-sized RNA with catalytic activity, it can cohere with reciprocal RNA substrate by base pairing, and dissect RNA substrate at special sites and clean up or block its express. Ribozyme has evident ascendant as a antisense drug, it has stable spatial structure and is not easily aggressed by RNA biocatalyst. Furthermore it can be used repeatly, it can brake old RNA and cohere with new RNA. In addition, ribozyme numerator is fairly diminutive inserted into gene therapy bearer easily. Hammerhead ribozyme which is known as minimum ribozyme can specifically concis target RNA with sequence and make target RNA express down regulation. This feature make it as a candidate in gene therapy.

Ribozyme can discriminate mutant or wild type RNA, and can specifically concis mutant gene transcript by gene P23H and S334Ter in vitro study, it can degrade protein level in anomal- erythropsin without impacting wild type proteinum, which is designed by Drenser and other researchers. Hammerhead ribozyme Rz10、Rz40、Rz9 and RzMM can reciprocal treat ADRP actively which is constructed by ONeill and other scientists and that can aim directly at human erythropsin catastrophe gene part and humanperipherin mutation gene part. This is the report that ribozyme can treat ADRP actively at the first time. Sullvan with others prove that hammerhead ribozyme treat ADRP actively in vitro study.

The ideal bearer to treat antisense gene

The safety, long-term, high performance and stabilization express genic bearer is the key point of genic therapy. Only that can bearer introduce the objective gene to amphiblestrodes and express stably. Virus is the most effective vivo bearer that we can get at present. Retrovirus, adenovirus and adeno-associated virus are frequently used. Every viral vector has different forte and deficient. Retrovirus has high efficiency of infection, lasting express and other characters. But the gene, which were introduced to, depends on cleavage of target cell. For terminally differentiated cells such as optic cell and retinal pigment epithelium it is difficult to transduction [9]. Adenovirus vector can not transfect chromatosome. exogenous gene is merely transient expression and it may cause severely inflammatory reaction and immune reaction, so adeno-associated virus is focused our attention upon as applied viral vector. Adenovirus vector is, as we know, the only vector that can conform to chromatin body without pathopoiesis and express stably. It can transfect proliferative cell and non-proliferative cell. It has a high transformation efficiency to photosensory cell and retinal pigment epithelium. It can not evoke evidently inflammation and immune reaction [13,14],because viral integration is stable. At equal time AAV takes along objective gene, it can express long time. For instance it can express 3 years in rat amphiblestrodes and 2 years in monkey amphiblestrodes without adverse reaction [15]. At present we consider that AAV introduce exogenous gene to photoreceptor cells effectively and express stably without pathogenicity, so it will became the most effective equipage in gene transfection. rAAV has not the deficient that bearer was olig-density at once upon time and that ADVs aid manufacture was not enough in the past, it can effectively stable mediate amphiblestrodes to transfer without evident toxicity and immune reaction [16].Its exogenous gene expression can last 18 monthes. The efficacy of transfecting photoreceptor cells in major creatures is higher 2000 times than AV. Different experimental animal model are injected subarachnoid space, gangliocyte and Beale's ganglion cells are transfected [17].The deficient of rAAV takes along DNA parts short (maximum 4.8kb), speed slowly, easily appears

gene mutation in the integration engineering and so on. Lewin with others [18] utilize rAAV bearer which has opsin promoter to mediate hammerhead ribozyme or hairpin ribozyme to rat amphiblestrodes cells. Through histopathology or ERG analyses, P23H trangenic mice that trouble ADRP delay cell degeneration at least 3 mouthes and conserve some visual function. LaVail with others does the same experiment. They displace ribozyme to degeneratio, pigmentosa retinae trangenic mice photosensor cell by rAAV. (Degeneratio, pigmentosa retinae is a dominant inherited disease fromed by erythropsin P23H gene mutation), mutational P23H mRNA is cutted. The result is that the photosensor cell degeneration of the mice can be delayed when injection was performed at postnatal 15 days (not still occurring evident degeneration) or 30 days and 45 days(40%~45% photosensor cells degenerescence),. This indicate that treatment utilizes after course of disease evolved at some stage.

Question

The desigin of ribozyme which dominant heredity diseases definite gene mutation generates has shortage that the different heterogenesis in the same gene can introduce the same ailment. Porphyropsin gene has over 100 heterogenesis that introduce ADRP. It is unrealistic that devises healing ribozyme. There is a ribozyme therapeutic regimen named ablation and commutation for dominant mutation wild type gene; wild-type gene. It proposes a concept "stable target site" that objective mRNA is cloned. Its enzyme cutting site removed by nonsense mutation. Ribozyme and "stable target site" are reprinted the same rAAV bearer or the same transcription element. Ribozyme is at 3untranslated region or at diverse transcription locations. This technique removes mutant and wild type mRNA and offer new wild stable heterogeny. In recent years this technique has been utilized to cure several allelic heterogeneity diseases [20,21].

Prospect

ADRP is a serious disease that is studied deeply. The success of ribozyme gene antisense treatment in the ADRP analogue means epoch will come that cure ADRP basically. The development of molecular biology will profit the gene antisense treatment in the ADRP applied to clinic subretinal space.

REFERENCES

1. Dryja TP, Hahn LB.Kajiwara K, and et al. Dominant and digenic mutations in the peripherin/RDS and ROM1 genes in retinltis pigmentosa. Inevst Ophthalmot Vis Sci,1997, 38: 1972~1982.
2. Mansergb FC, Millington-Ward S, Kennan A, and et al. Retinitis pigmentosa and progressive sensorineural hearing loss caused by a C12258A mutation in the mitochondrial MTTS2 gene. Am J Hum Genet,1999,64:971-985.
3. Pagon RA, Daiger SP, Reitnitis pigmentosa overview. http://www.geneclinics.org.
4. http//utsph.sph.uth.tmc.edu/www/Wtsph/RetNet.
5. Li T, Sandberg MA, Pawlyk BS, and et al. Effect of vitamin A supplementation on rhodopsin mutants threonine-17 hmethionine and praline-347 ichserine in transgenic mice and in cell cultures. Proc-Natl-Acad-Sci-U-S-A, 1998, 95: 11933-11938.
6. Frasson M, Picaud S, Leveillard T, and et al. Glial cell line-derived neurotrophic factor induces histologic and functional protection of rod photoreceptors in the rd/rds mouse. Invest-Ophthalmol-Vis-Sci, 1999, 40:2724-2734.
7. Larkin DF, Alexander RA, Cree IA. Infiltrating inflammatory cell phenotypes and apoptosis in rejected human corneal allografts. Eye, 1997, 11: 68-74.
8. The transplantation of human fetal neuroretinal cells in advanced retinitis pigmentosa patients: retinitis of a long-term safety study.
9. Pepose JS, Leib DA. Herpes simplex viral vectors for therapeutic gene delivery to ocular tissues.Recent-breakthroughs in the molecular genetics of ocular diseases[J].Invest Ophthalmol Vis Sci, 1994, 35:2662-2666.
10. Kimberly A. Drenser, Adrian M. Timmers, William W. Hauswirth2 and Alfred S. Lewin Ribozyme-targeted destruction of RNA associated with autosomal-dominant retinitis pigmentosa. Invest-Ophthalmol-Vis-Sci, 1998, 39:681-689.
11. ONeill B, Millington-Ward S, OReilly M, and et al. Ribozyme-based therapeutic approaches for autosomal dominant retinitis pigmentosa. Invest-Ophthalmol-Vis-Sci, 2000, 41: 2863-2869.
12. Sullivan JM, Pietras KM, Schlichtenbrede FC, and et al. Hammerhead ribozymes designed to cleave all human rod opsin mRNAs which cause autosomal dominant retinitis pigmentosa. Mol-Vis,2002, 8:102-113.

13. Wang F, Rendahl KG, Manning WC, and et al. AAV−mediated expression expression of vascular endothelial growth factor induces choroidal neovascularization in rat. Invest − Ophthalmol−Vis−Sci, 2003, 44:781−790.
14. Hauawirth WW, Li Q, Raisler B, and et al. Range of retinal diseases potentially treatable by AAV−vectored gene therapy. Novartis Found Symp.2004, 255:179−88;discussion 188−94.
15. Hauswirth WW, Beaufrere L. Ocular gene therapy: Quo vadis? Invest−Ophthalmol−Vis−Sci. 2000, 41: 2821−2826.
16. Bennett J, Duan D, Engelhardt JF, and et al. Real−time, noninvasive in vivo assessment of adeno−associated virus−mediated retinal transduction. Invest Ophthalmol Vis Sci, 1997, 38: 2857−2863.
17. Dreyer EB, Voruerk CK, Zurakouski D, and et a1.Inflection with adeno−associated virus may protect against excite toxicity. Neuroreport, 1999, 10:2887−2890.
18. Lewin A.S. et al. Ribozyme rescue of photoreceptor cells in a transgenic rat model of autosomal dominant retinitis pigmentosa. Nature, 1998, 967−971.
19. LaVail MM, Yasumura D, Matthes MT, and et al. Ribozyme rescue of photoreceptor cells in P23H transgenic rats: long−term survival and late−stage therapy. Proc−Natl−Acad−Sci−U−S−A, 2000, 97: 2863−2869.
20. Zem M.A. and et al. A novel SV40−based vector successfully transduces and expresses an 1−antitrypsin ribozyme in a human hepatoma−derived cell line. Gene Ther, 1999, 6:114−120.
21. S.Millington−Ward et al. Strategems invitro for gene therapies directed to dominant mutations. Hum.Mol.Genet. 1997, 6:1415−1426.