Gene Transfer into Schistosome as a Therapy Tool

Hongbao Ma, George Chen

Department of Medicine, Medical School, Michigan State University, East Lansing, MI 48824, USA
hongbao@msu.edu; 517-432-0623

Abstract: The pathological changes caused by blood fluke schistosomes are caused by both female and male parasitism in the host body. The eggs produced by fertilized female schistosomes are main pathogenic resource. The male worms alone lives in the host that do not result to the clinical symptoms, as there is no side product from this infection if there are only male worms in the vessels. In general, the male schistosome parasites can live in host vessels for up to 30 years. It will be possible to establish a model to transfer clinically valuable gene(s) such as human insulin gene into schistosome, select the positive transfected male worms, set up the transgenic male worm into animal veins, and to observe the expression level of the gene(s). Schistosome could reside in the mice, and Schistosoma mansoni could be generated in vitro by transforming miracidia. The snail Australorbis glabratus is the host of Schistosoma mansoni, which could be maintained in the spring water or artificial spring water in the plastic container and fed once or twice a week on lettuce. Snail eggs or small snails can be removed from the container to hold down the size of population. After infected with miracidia, cecariaes are collected after 45 days infection for the animal infection. Genes of interest (such as human insulin gene) can be introduced downstream of in situ where either green fluorescent protein or luciferase are used as reports. Active promoter of heat shock protein 70 of Schistosoma mansoni can be utilized in this strategy. Gene gun or other gene transfection methods (such as laser gene transfer, heat enhanced gene transfer, lipofection and calcium phosphate coprecipitation) can be used for gene transfer. West blotting and ELISA will be used for the gene expression detection. Genomic DNA can be isolated from the transfected worms and PCR or Southern blotting will be used to confirm the transfection. The schistosome with stable transfection can be screened in vitro and in vivo to find the worms carrying target gene in the next generation. This will provide a novel way for the efficient and safety gene therapy, and it will be benefit millions of patients after the real clinical trial in the future. [Nature and Science. 2004;2(4) (Supplement): 8-16].

Keywords: cecaria; gene transfer; miracidia; schistosome

1. Introduction
Gene therapy has reached a crossroad during the past years (Matsui, et al., 2003). Gene therapy can be defined as the deliberate transfer of DNA for therapeutic purposes. The concept of genetic information transfer as a practical clinical tool arose from the gene cloning technology developed during the 1970s (Bechtel, et al., 1979). There is a further implication in that it involves only specific sequences containing relevant genetic information. Transplantation procedures involving bone marrow, kidney and liver are not considered a form of gene therapy. Without the ability to isolate and replicate defined genetic sequences it would be impossible to produce purified material for clinical usage. The drive for the practical application of this technology came from the biotechnology industry, with its quest for complex human biomolecules produced by recombinant techniques in bacterial. Within a decade, pharmaceutical-grade insulin, interferon, interleukin-2 (IL-2) and tumor necrosis factor (TNF) were all undergoing clinical trials. The next step is to obtain gene expression in vivo. Genetic disorders are the obvious first target for such therapies. Abortive attempts were made in the early 1980s to treat two patients with thalassaemia (Temple, et al., 1982). These experiments were surrounded by controversy as the pre-clinical evidence of effectiveness was not adequate and full ethical approval had not been given. For the features of a suitable target disease for gene therapy approaches, certain factors should be considered. The disease must be life threatening so that the potential risk of serious side effects is ethically acceptable. The gene must be available and its delivery to the relevant tissue feasible. This may involve the ex vivo transfection or transduction of cells removed from a patient, which are returned after manipulation. This approach is only possible with a limited range of tissues and most trials so far have used bone marrow. Ideally, a short-term surrogate end-point to demonstrate the physiological benefit of the newly inserted gene should be available. The electrical conductance change in the nasal epithelium after insertion of the cystic fibrosis trans-membrane regulator gene is a good example. Finally, there must be some possibility that the
disability caused by a disease is reversible. Some of the tragic mental and physical handicaps caused by genetic metabolic disorders may never be improved by somatic gene therapy, however successful with a gene transfer protocol. Gene transfer is one of the key factors in gene therapy. In this project, we will use gene gun or other methods (such as calcium phosphate coprecipitation, lipofection, laser or temperature enhancing gene transfer) as the tools to transfer insulin and other proper genes into schistosome.

The blood fluke schistosomes are unusual trematodes that reside in the blood vessels of the definitive host. There are a number of species of schistosomes that can infect humans, but most human infections are caused by one of the three following species: *Schistosoma mansoni*; *Schistosoma haematobium* and *Schistosoma japonicum*. Even it is estimated that approximately 200,000,000 people are infected with schistosomes, resulting in 1,000,000 deaths each year (Parasites Research Group, 2004), the disease from schistosomes arose by only the female and male together in the body, and male worms only do not arouse symptom. If there are only male schistosome parasites in animal vessels, they can live in animal vessels for up to 30 years without symptom, as there is no side product from this infection if there are only male worms in the vessels.

The cultured sporocysts of schistosomes have been transiently transfected with pBluescript-based plasmids expressing green fluorescent protein (GFP) (Wippersteg, et al., 2002a; Wippersteg, et al., 2002b), demonstrating that it is possible in principle to generate transgenic parasites. However, it is not clear yet whether a transgenic line of schistosomes could be established from these transiently transfected sporocysts. At least two important obstacles on the path to the development of a reliable, tractable transgenesis system for schistosomes now need to be surmounted: (1) development of appropriate constructs to achieve stable integration into the schistosome genome and (2) a demonstration that the life cycle can be completed by genetically modified parasites.

The life cycle of the schistosome includes two free-living, self-reliant, and mobile infective stages. These are the miracidium, which seeks out and directly infects the intermediate host snail, and the cercaria, which accomplishes infection of the mammalian, definitive host by direct penetration of host skin when a definitive host comes into contact with water contaminated with cercariae. These are the only two stages that could be reintroduced into the life cycle using a natural route of infection, thereby obviating the inefficient and laborious tasks of surgically implanting transformed sporocysts back into snails and/or surgically implanting adult schistosomes into the portal system blood vessels of mice (Cheever, et al., 1994; Jourdane, et al., 1985).

Here we subject miracidia of *Schistosoma mansoni* to particle bombardment and are able to show that miracidia bombarded with DNA-coated gold particles can directly and naturally infect the intermediate host snail *Biomphalaria glabrata* and establish as sporocysts in a natural fashion. Further we are able to demonstrate transgene transcription of enhanced GFP in adult worms infected with transformed sporocysts.

Insulin gene will be considered as one of the targets in this project. Diabetes is a disease in which the body does not produce or properly use insulin. Insulin is a hormone that is needed to convert sugar, starches and other food into energy needed for daily life. There are 18.2 million people in the United States, or 6.3% of the population, who have diabetes (American Diabetes Association, 2004). The treatment of diabetes is one of the most important topics in the health science. The normal treatment of diabetes is to inject insulin to patients every day to keep the proper insulin level in the blood. It is high cost and plague. The genetic treatment has not been successfully. If it successfully transfers insulin gene into schistosome and the gene successfully expresses in the worm, and infect the male transected worm in animal vessels, the worm can release suitable amount insulin into animal vessels. This will be a high efficient treatment method on the diabetes. The potential valuation of this project for diabetes treatment is to clone the insulin gene (with prometer) into schistosome and select male worm, then reside the cloned schistosome to diabetes patients’ vessels.

2. Specific Aims this Trial can Hit

The scientific objective of this scientific trial is to establish an animal model by using transgenic schistosome as an alternative therapeutic material delivery vehicle for animal and eventually for human in the future.

2.1 To select one or a group of candidate genes, such as insulin, thyrotropin, growth factor and tumor necrosis factor genes, reconstruct the selected genes and transfec them into schistosome by the gene gun, temperature enhanced gene transfection, or laser enhanced gene transfection, etc.

2.2 To infect the transgenic schistosome in mice and valuate the transfected gene expression in the mice.

2.3 To establish the transgenic schistosome model and evaluate the medication capacity and possible expression level control.

2.4 Elongating knowledge from aims 1 – 3 for the future project, to explore the possibility of infecting the transgenic schistosome in human and evaluate the
potential clinical valuations and problems from the transgenic schistosome.

The pathologic changes caused by blood fluke schistosomes are caused by both female and male parasitism in the host body. The eggs produced by fertilized female schistosomes are main pathogenic resource. The male worms that live in the host alone do not result to the clinic symptoms. The proposed studies address an area of the critical trial to deliver materials by the expression of genes cloned in male schistosome that can live in human bodies up to 30 years without clinical syndrome. After maturing the gene transfection technique of schistosome, it will provide a totally new, efficient, economic and safety way in the material delivery, which is supposed to be applicable in curing diseases such as diabetes, thyroid hypofunction or cancer.

3. Related Studies by the Authors
There were many successful studies for the gene transfer and its detections in the past decades. Here it gives some related studies the authors of this article did as the technique and theory references of gene transfer into schistosome as a therapy tool. All the techniques are available for the ideas in this article.

3.1 Increased temperature enhanced gene transfer
The heated cultured human aorta smooth muscle cells had a significantly higher expression of the transfected swine growth hormone gene. When the swine growth hormone gene and human smooth muscle cells were incubated under the various temperatures of 23°C, 37°C and 43°C, the transfection increased with the temperature elevation (p<0.01) (Figure 1A). The greatest effects occurred within 10 min of incubation and persisted up to 30 min (Ma, et al., 2004a). In another experiment, we got the same result to transfer human interleukin-2 gene into cultured rat myocytes (Ma, et al., 2004b) (Figure 1B). These results suggest that even a few degrees of ambient temperature rise can significantly increase gene transfer into cells. This may be of value when using gene therapy with transfection procedures.

3.2 Gene transfection mediated by lipofection
With lipofection method (Lipofectamine Reagent, #18324-012, Invitrogen Corporation, Carlsbad, CA), we successfully transferred GFP gene into WB-S cells and expressed the GFP gene in the WB-S cells (Figure 2). This lipofection gene transfer method will be an alternative choice in the gene transfer execution in this project.

3.3 Laser Enhanced Gene Transfer
UV excimer laser (XeCl2, 308 nm excimer laser, Spectranetics CVS-300™, Spectranetics, Colorado Springs, CO) was used with a 2.0 mm diameter optical fibers for the gene transfer. Human aorta smooth muscle cells were cultured in F12K medium containing 2 mM glutamine, 10 mM HEPES, 10 mM TES, 50 ng/ml ascorbic acid, 10 μg/ml insulin, 10 μg/ml transferrin, 10 ng/ml sodium selenite and 30 μg/ml endothelial cell growth supplement, FBS 10% (Gibco BRL Life Technologies, Inc., Grand Island, NY). Gene labeled with anti-ampihilene mutation was used. The results showed that laser enhanced the gene transfer. This laser gene transfer method will be used in the gene transfer in this project.

3.4 Gene gun enhanced gene transfer
With the self-design CO2 propelled gene gun (200 psi, distance 3 cm with 400 mesh nylon screen) using tungsten particle (600 nm diameter) coated with plasmid expressing anti-ampicillin gene, the plasmid with anti-ampicillin gene was high efficiently transferred into E. Coli cells. This gene gun technique will be used in this project for gene transfer.
3.5 C-reactive protein measurement by ELISA

Elevated levels of C-reactive protein (CRP) have been associated with increased risk for development of cardiovascular events. In order to follow the trend of CRP over the course leading to an acute event, we evaluated CRP levels under three conditions: normal rabbits, atherosclerotic rabbits before and after pharmacological triggering of plaque rupture and thrombosis. Rabbit atherosclerosis was induced with balloon deendothelialization and feeding a high cholesterol diet for 9 months. Plaque rupture and thrombosis were induced using Russell viper venom (RVV) and histamine with the atherosclerotic rabbit model. Serum samples were obtained from control rabbits (n=3), and atherosclerotic rabbits, before (n=6) and 48 hours after RVV and histamine-induced thrombosis (n=8). Rabbit specific high sensitivity ELISA was developed to detect the levels of serum CRP concentrations. CRP levels were significantly lower in control rabbits compared to rabbits with atherosclerotic plaques. The results further demonstrated that rabbits with RVV and histamine-triggered thrombosis had significantly higher levels of serum CRP than non-triggered atherosclerotic rabbits (Figure 3) (Ma, et al., 2004c). The rise of serum CRP levels both after cholesterol feeding and pharmacological triggering of thrombus may help using of CRP to evaluate not only the long-term risk but also a short-term risk of events if CRP levels increase acutely. The ELISA method will be used in the gene expression detection of this project.

Figure 3. The effect of thrombosis on rabbit serum C-reactive protein (CRP) expression, measured by ELISA (Ma, et al., 2004c).
3.6 Heat shock protein 70 measured by Western blotting

Transmyocardial laser revascularization (TMLR) has been shown to relieve symptomatic ischemia but laser tissue effects have potential complications. In order to define the mechanism of laser action, heat shock protein (hsp) expression was evaluated in rat hearts after TMLR. Under general anesthesia, hearts were removed from 10 rats and immediately placed in oxygenated physiologic buffered solution (PBS) at 0°C. After the various treatments, hearts were homogenized and hsp70 was measured with Western Blotting. Group 1 (n=3) hearts were immediately homogenized; Group 2 (n=3) hearts were perfused with the PBS in a Langendorff setup for 6 h; Group 3 (n=3) hearts were lased (50 channels) using a Ho:Yag laser via a 0/600 mm core fiber at 3 Hz and 280 mJ/pulse and perfused up to 6 h. Group 4 (n=1) rat was heated to 42°C for 15 min then recovered at 23°C for 6h prior to hsp measurement. There was a significantly lower hsp70 expression in Group 3 (TMLR) and higher in Group 4 than that the control Groups 1, 2 (Table 1) (Ma, et al., 2004d). The Western Blotting technique will be used in the gene expression detection.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group 1*</th>
<th>Group 2*</th>
<th>Group 3**</th>
<th>Group 4</th>
</tr>
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<tbody>
<tr>
<td>Relative OD_{600nm}</td>
<td>1</td>
<td>0.87±0.10</td>
<td>0.19±0.03</td>
<td>3.47</td>
</tr>
</tbody>
</table>

* to *: p=ns; * to **: p<0.003

4. Research Design and Methods

The principle idea of this project is to insert target genes into the chromosome of schistosome eggs (single cell stage) to get stable gene transfection in the single egg cell. After then, raise the male transfected worms in animal and let the target gene be expressed in animals. This will provide an alternative treatment of genetic diseases with the transfected gene(s).

4.1 The brief description of this project steps is as the following:

4.1.1 To get target gene candidate(s) with promoter, plasmid, and expression control sequence.
4.1.2 To construct the expression plasmids with target genes (with suitable promoter).
4.1.3 To transform the expression plasmid(s) into schistosome eggs, miracidium or cercariae.
4.1.4 To detect the gene product if it is expressed in miracidium and cercariae.
4.1.5 To raise the transformed schistosome in mice.
4.1.6 To investigate the expression level of the target gene in the mice infected by transformed schistosome.
4.1.7 To screen the male schistosome transformed with target gene(s), and culture the male schistosome in animals.
4.1.8 To observe if the male schistosome can survive in the animal without hurting the animal and express the target protein.
4.1.9 To keep the schistosome transfected with target gene(s).
To select the male schistosome transfected with target gene(s).
4.1.10 To raise the male schistosoma transfected with target gene(s) in animal.
4.1.11 To observe if the male schistosome can survive in the animal without hurting the animal and express the target protein.
4.1.12 Complete the model to transfer valuable genes into schistosome for the material delivery.

4.1.13 To transfer gene efficiently, a novel method gene-gun will be used as the first choice. The other gene transfection methods including laser gene transfer, temperature enhanced gene transfer, electroporation, lipofection, calcium-phosphate-mediated coprecipitation, transfection mediated by DEAE-dextran, etc. will be considered.

The transfected gene expression can be controlled by regulating the transgenic schistosome number, etc.

Genes of interest (such as human insulin gene) can be introduced downstream of in situ where either green fluorescent protein or luciferase are used as reports. Active promoter of heat shock protein 70 of *Schistosome mansoni* (GenBank No. L02415) can be utilized in this strategy. Gene gun (Bio-Rad Laboratories) or other gene transfection methods (such as laser gene transfer, heat enhanced gene transfer, lipofection and calcium phosphate coprecipitation) can be used in this project for gene transfer. West blotting and ELISA can be used for the gene expression
detection. Genomic DNA can be isolated from the transfected worms and PCR or Southern blotting can be used to confirm the transfection. The schistosome with stable transfection can be screened in vitro and in vivo to find the worms carrying target gene in the next generation.

4.2 Maintenance of Schistosoma mansoni

Schistosome will reside in mice. Schistosoma japonicum and Schistosoma mansoni sporocysts can be generated in vitro by transforming miracidia (Coustau, et al., 2000).

The snail Australorbis glabratus is the host of Schistosoma mansoni, which is maintained in the spring water or artificial spring water in the food storage plastic container. Feed once or twice a week on lettuce. Temperature should be maintained above 25°C. Snail eggs or small snails can be removed from the container to hold down the size of population. Schistosoma mansoni adults are best maintained in mice or hamsters.

4.3 Obtaining miracidia

Remove the livers of 1-4 infected animals. Homogenize the liver 20-30 seconds in 0.85% NaCl in blender. Pour homogenate into a flask and allow to settle for 10-20 min. Repeat above procedure 2-3 times until supernatant appears clear. Pour off final supernatant. Fill the flask nearly to the top with water. Place flask in bright light for 5 min to stimulate hatching of miracidia. Collect miracidia from the top of flask and transfer to a small dish for infecting snails.

4.4 Infecting snails

Keep snails in small amount of spring water in small beakers. Add miracidia. For routine maintenance five miracidia per snail gives a high incidence of infection. Infected snails will usually be shedding cercariae in 30 days.

4.5 Obtaining cercariae

To obtain large number of cercariae, place infected snails in the dark for 2-5 days before release cercariae. To check incidence of infection, isolate snails in separate beaker with spring water. Use rubber gloves and scoop. Place 10-20 snails in a beaker with 100-200 ml of spring water. Place beaker in strong light; cercariae will emerge in 5-60 min. Inspect beaker with a dissecting scope.

4.6 Infecting mice or hamster

Wear gloves. Take 0.1 ml aliquots of the cercarial suspension, dilute the suspension with spring water to the desired number of cercaria in 0.2 ml spring water. Inject 50-200 cercariae into the abdomen of mouse or hamster. Mature worms develop in about 40 days. Feces may be examined for the presence of eggs to determine presence and maturity of infection. Adult worms can be recovered by dissection from the mesenteric veins.

4.7 Gene construction

To date, stable transformation has not been achieved in any schistosome. While transient transfection provides a useful tool to study the effects of over expression of genes of interest. To effectively generate transformants, the appropriate vector/promoter combination must be chosen to ensure maximum expression. Active promoter of heat shock protein 70 (hsp70) of Schistosome mansoni (GenBank No. L02415) (Neumann, et al., 1992) will be utilized in this strategy. Genes of interest (such as insulin gene) will be introduced downstream of in situ where either green fluorescent protein or luciferase are used as reports. Therefore, we will use pUC19 plasmid to deliver our current combinations of genes into the candidate worms.

Briefly, target genes will be constructed into gene-promoter-vector as the following:

To engineer plasmid constructs for this transfection, polymerase chain reaction (PCR), TOBO (which is used for cloning after obtain a PCR product with a TA over hung) and Bluescript/plasmid (Invitrogen Corporation, Carlsbad, CA) is used for DNA fragment amplification as well as pUC19 plasmid backbone or a retroviral vector, G1Tk1SvNa vector (Sauce, et al., 2002) are used for gene transfection.

According to sequence data, gene primers are designed to amplify parts of the gene by PCR. The restrictions sites are used to insert the gene fragment by ligating the fragment into pUC19 plasmid. The clones containing the promoter (such as CMV) enhanced green fluorescing protein as biological marker, and terminator regions will be inserted:

4.8 PCR and RT-PCR analysis

Amplification reactions are performed in a total volume of 25 ml using 10 ng DNA isolated from worms for genotyping. 1 mM each primer gene forward and the gene reverse primer, 10 mM of each deoxynucleotide and 2.5 units Taq polymerase (Invitrogen Corporation, Carlsbad, CA). After an initial denaturation step at 95°C for 3 min, temperature cycling is performed at 93°C-45 seconds, 60°C-60 seconds, 70°C-45 seconds for 30 cycles. One fourth of the reaction volume is used for agarose gel electrophoresis (1.2%). Reverse transcriptase-polymerase chain reaction (RT-PCR) used
for gene expression evaluation and will be done stepwise in separate reactions.

4.9 Commercial gene

Insulin gene cloned in E. Coli and promoter could be obtained from American Type Culture Collection (ATCC, Rockville, MD). This insulin gene and promoter will be used to transfer to schistosome. Other suitable genes for the transfection will be considered during the processing of this project.

4.10 Gene transfer

To attain the maximal transformation efficiently, the gene gun method will be used as the first choice for the gene transfection. Bio-Rad gene gun and a self-design gene gun will be used for the gene transfection. Also, a rifle gun will be used as an alternative method for the gene transfection. Other gene transfer methods will be considered.

4.10.1 Gene gun transfection

_Schistosoma mansoni_ will be propagated in the laboratory as described (Copeland, et al., 2003). Snails are maintained at 26°C in aerated water and fed with lettuce leaves, in a room with a light/darkness cycle of 12 hours each. At 8 weeks after _Schistosoma mansoni_ infection, mice are killed, adult worms are perfused from the mesenteric veins with PBS, and the livers are removed. Adult worms are washed three times in RPMI 1640 and maintained _in vitro_ in RPMI 1640 + 10% FCS supplemented with penicillin/streptomycin at 37°C in 5% CO₂. The infected livers are forced through a plastic membrane (Transwell, Costar), water or medium is collected from the water, and concentrated by centrifugation at 500 rpm. For particle bombardment (biolistics), approximately 500 miracidia or 20 male, adult worms are evenly spread onto a polycarbonate mesh to release the schistosome eggs, after which the eggs are washed in cold sterile 1.8% NaCl to remove host tissues and debris. The 1.8% NaCl is replaced with sterile water to induce the eggs to hatch, miracidia are collected from the water, and concentrated by centrifugation at 500 rpm. For particle bombardment (biolistics), approximately 500 miracidia or 20 male, adult worms are evenly spread onto a polycarbonate membrane (Transwell, Costar), water or medium is removed and target schistosomes on the polycarbonate membranes are positioned in a Biolistic PDS-1000/HE Particle Delivery System (Bio-Rad Laboratories GmbH, München, Germany). Biolistic parameters are 15 in. Hg of chamber vacuum, target distance of 3 cm (stage 1), 900 psi (miracidia) or 1800 psi (adult worms) particle acceleration pressure, and 1.0 μm gold microcarriers (Bio-Rad). Gold microcarriers are prepared, and circular plasmid DNA is precipitated onto the gold using methods recommended by Bio-Rad with the following modification; 0.6 mg of gold particles carrying ~5 μg of plasmid DNA is used per bombardment.

4.10.2 Laser transfection

UV excimer laser (XeCl₂, 308 nm) will be used in the gene transfection (5 min by a 0.7 0.9, 1.4 or 2.0 mm diameter fiber with fluence of 45 and 60 mj/mm² - real laser energy 2.3, 5.9, 13.1, 32.0 mj/pulse, 25 Hz) (CVX-300 Excimer Laser System, The Spectranetics Corporation, Colorado Springs, CO, USA). Nd:Yag, Ho:Yag will be considered as other candidates. The laser equipments are available in PI’s lab.

4.10.3 Other transfection methods

For the alternative, other transfection methods will be considered, such as lipofection (Young, et al., 2002), heat enhanced gene transfer (Ma, et al., 2004a; Ma, et al., 2004b), calcium phosphate coprecipitation methods will be considered (Sambrook, et al., 1989; Ausubel, et al., 1992), electroporation, and DEAE-dextran transfection (Puchalski and Fahl, 1992).

4.11 Screening and confirmation of transformed worms

West blotting and ELISA will be used for the gene expression detection (Ma, et al., 2004c; Ma, et al., 2004d). Genomic DNA will be isolated from the transfected worms and PCR or Southern Blotting will be used to confirm the transfection (Spielmann, 2002).

4.12 Vertebrate animals

Five hundred C57BL/6 mice (Charles River Laboratories, Inc., Wilmington, MA, USA) will be needed as the schistosome host in this project. Mice are housed according to NIH guidelines and the study is conducted according to Michigan State University’s Animal Care and Use Committee approved protocol.

4.13 Statistical analysis

With Microsoft Office Excel and Jandel Scientific program SigmaStat (Sigma Chemical Co., St. Louis, Missouri) can used for data statistical analysis of transfected gene expression data. P<0.05 is considered statistically significant difference. Measured data are reported as mean±SD. The student t-test is used for comparison.

5. Discussions

The Food and Drug Administration has not yet approved any human gene therapy product for sale. After get the matured method to transfer genes to schistosome and safety raise the transformed male schistosome in animal vessels, and get the expression product release of the transfected gene in the animals, this project will provide a totally new, efficient, economic and safety way in the material delivery and gene therapy to cure diseases such as diabetes, thyroid hypofunction or cancer, and it will be benefit millions of patients after the real clinical trial in the future. And, it will open a totally new way for the gene therapy.
Also, the achievement of this project will be benefit in the veterinary application and agriculture applications.

The proposed studies address an area of the critical important trial to cure diabetes with the human insulin gene cloned in male schistosoma that can live in human bodies up to 30 years and without physical hurt for human, or it can provide the treatment of other diseases with other suitable genes transfected. This project is supposed to get a safety and economic way to cure diabetes or other diseases efficiently.

This is the first suppose trying to practice using schistosoma as the gene therapy tool in the world. This can make a universal therapy tool and provide a more effective and safety way for gene therapy. It will improve both life science research and clinical practice.

Correspondence to:
Hongbao Ma
B410 Clinical Center
Michigan State University
East Lansing, MI 48824, USA
Telephone: (517) 432-0623
Email: hongbao@msu.edu

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