

Nuclear Transfer and Human Genome Therapy

a report by

Dr Peter K Law

Founder, Chairman and Chief Executive Officer, Cell Therapy Research Foundation (CTRF)

Dr Peter K Law is the Founder, Chairman and Chief Executive Officer of the Cell Therapy Research Foundation (CTRF) and also Cell Therapy, Inc. Founded in 1991, CTRF focuses on developing biologic therapies in treating hereditary, debilitating and fatal diseases. He pioneered and holds world patent rights to myoblast transfer therapy, the only human genome therapy in existence. A former Professor of Neurology in The University of Tennessee, Memphis and Vanderbilt University, Dr Law received his BSc with honours from McGill University, MSc and PhD from the University of Toronto and Postdoctorate qualifications from McMaster University in Canada.

Introduction

The cell is the origin of all life. Contained within its nucleus are more than 30,000 genes that determine cell normality and cell characteristics.¹ The genes are composed of deoxyribonucleic acid (DNA) that is spatially and temporally switched on and off during development to produce more than 100,000 different transcripts of ribonucleic acid (RNA). The transcriptional events occur inside the nucleus and require the nuclear matrix and/or the chromatin to operate efficiently. These regulatory events are poorly understood but invariably involve polygenic interactions.

While the basic sequence of the human genome has been determined, as a result of significant effort and expenditure, the understanding of how the genome actually functions will take many decades of further research. Scientists do not know the spatial and temporal interactions of the RNA transcripts and know little of their modes of action. Numerous methods have yet to be developed to determine the diverse functions of some 30,000 genes and more techniques have to be refined to effect gene regulation and expression. It is through this knowledge that pharmacogenetics may one day provide rational approaches in therapeutics. Today, no genetic treatment has been developed based on the Human Genome Project. The analysis of DNA/RNA variations and gene expression are used mainly in diagnostics, while gene therapy success through single gene manipulation has been rare.

Myoblast Transfer Therapy (MTT)

An alternative perspective is that a genetically abnormal cell degenerates due to the lack of the normal genome. In hereditary degenerative diseases such as muscular dystrophies, the much-needed normal genome can be incorporated into the

dystrophic muscle fibres. This is achieved by taking a muscle biopsy from normal donors, culturing pure embryonic muscle cells called 'myoblasts' and injecting the normal myoblasts into dystrophic muscles. This cell transplant procedure is called myoblast transfer therapy (MTT).

Through natural cell fusion, which is inherent in myogenesis and muscle regeneration, the donor myoblasts insert their normal nuclei that contain the human genome into the dystrophic muscle fibres, forming multinucleated heterokaryons to effect genetic complementation repair.² The donor nuclei operate to transcribe the missing RNA.

Only transfer of the normal nuclei, carrying the genomic softwares and the chromosomal hardwares, will allow the orderly provision of various co-factors necessary for the regulation and the expression of the transgene.³ Natural transcription of the normal genome within the donor nuclei following MTT ensures orderly replacement of any protein deficiency resulting from single gene defects or from haphazard polygenic interactions, much of which is unknown. This differs significantly from single gene transduction, effected through viral or non-viral vectors, in that the transgene may find no transcriptional factors/co-factors in the adult environment for its regulation and expression. Many of these co-factors are the products of other genes that are only operative in early development.

Unique Biotechnology

MTT is a platform technology of cell transplantation, nuclear transfer, gene therapy and tissue engineering. It is the only human genome therapy in existence, and will remain so until another modality is discovered to deliver the human genome into the defective cells of a genetically ill patient. Myoblast is the only somatic cell type that has the ability of

1. J C Venter, et al., "The Sequence of the Human Genome", *Science*, 291 (2001), pp. 1,304-1,351.
2. P K Law, et al., "Dystrophin Production Induced by Myoblast Transfer Therapy in Duchenne Muscular Dystrophy", *Lancet*, 336 (1990), pp. 114-115.
3. P K Law, "Myoblast Transfer as a Platform Technology for Gene Therapy", *Regulatory Affairs Focus*, (Technology), 4 (1999), pp. 25-27.

natural cell fusion. MTT is uniquely suited to treat hereditary muscle degeneration and weakness through nuclear transfer or genome transfer.

When donor myoblasts fuse among themselves after MTT, they form new muscle fibres to repopulate the degenerative organ, depositing contractile filaments to augment its function. Thus, as a cell therapy, MTT applies not only to all forms of skeletal muscle degeneration, but to heart muscle degeneration, body-building, anti-ageing and soft-tissue enhancement.⁴

MTT replenishes degenerated cells through cell therapy, and repairs degenerating cells through genome therapy. First conducted in February 1990 and published on 14 July 1990, MTT is the world's first human gene therapy.¹

An Enabling Technology

Approximately 230 MTT procedures have been conducted in the past 12 years on patients suffering from Duchenne, Becker or Limb-Girdle muscular dystrophies. There has not been a single death or coma or failure in heart, lung, kidney or liver function related to MTT. Expected adverse reactions include mild fever (<101°F), pain, nausea and/or erythema lasting up to three days. Two months of immunosuppression is sufficient to prevent rejection of the foreign myoblasts because mature muscle fibres do not express MHC-1 surface antigens. Therapeutic gene expression is efficient and stable, lasting up to six years after MTT.⁵

Phase II clinical trials had provided significant safety and efficacy data that the US Food and Drug Administration (FDA) has approved direct cost recovery, and encouraged the Cell Therapy Research Foundation to initiate Phase III multicentre clinical trials. The study is currently on hold pending resolution of documentation and current good manufacturing practice (cGMP) issues.

Heart Cell Therapy

There is considerable excitement about myoblast therapy restoring tissue in heart patients, with heart-muscle degeneration being the leading cause of debilitation and death in humans.^{6,7} Since the 1990s,

various laboratories have demonstrated the safety and efficacy of injecting cultured autologous myoblasts into infarcted hearts of animals. In May 2000, human myoblasts were delivered percutaneously and endovascularly with intramyocardial injections in a swine heart to determine the safety and feasibility of heart cell therapy (HCT), which aims to repopulate the dying heart with live muscle cells, increasing heart contractility, thus improving the quality of life and lengthening the lifespan of heart patients.

Cells were injected through a catheter threaded through the femoral artery into the left ventricle of the heart. Approximately one billion human myoblasts were injected through a needle timed to protrude six millimetres from the tip of the catheter into the heart muscle. Twenty injections, with volumes of 0.1ml, 0.2ml, 0.3ml, 0.5ml or 1.0ml, were made at cell concentration of 100 million per ml. There were no significant changes in heart rate, electrocardiogram and body temperature throughout the experiment. Passage through the injection catheter caused less than 5% cell death.⁸ At the completion of the procedure, the heart was processed for examination. There was no perforation of the injected heart. Human myoblasts were found widely and evenly distributed throughout the myocardium where the cells were injected.

This initial human myoblast transfer into the porcine heart delineates safety parameters of cell number, cell concentration, injection volume and delivery method. Human HCT has begun since then and, by now, more than 13 patients suffering from congestive heart failure have received myoblast injections in conjunction with open-heart bypass surgery in France and the US.

One patient has received myoblasts through a catheter. Reportedly, two patients have died unrelated to the myoblast administration, and the rest are in a stable condition. Some have even demonstrated improved heart function since myoblast transfer.⁹ All patients used their own myoblasts as autografts. HCT is a new frontier of MTT. It makes the patient's own muscle cells available to repopulate the infarct, secreting the various regenerative factors and depositing contractile proteins to regain heart function.

4. P K Law, et al., "Myoblast Transfer as a Platform Technology of Gene Therapy", *Gene Therapy & Molecular Biology*, 1 (1998), pp. 345-363.

5. P K Law, et al., "First Human Myoblast Transfer Therapy Continues to Show Dystrophin After 6 Years", *Cell Transplantation*, 6 (1997), pp. 95-100.

6. R Winslow, "Cell Therapy Restores Tissue in Heart Patient", *The Wall Street Journal*, 13 Nov 2000.

7. S Sternberg, "Cell Transplant Saves Dying Heart", *USA Today*, 13 Nov 2000.

8. P K Law, et al., "World's First Human Myoblast Transfer into the Heart", *Frontiers in Physiology*, (2000), p. A85.

9. P Menasche, et al., "Myoblast Transplantation for Heart Failure", *Lancet*, 357 (2001), pp. 279-280.

Myoblast Manufacture and Transplants

The holder of the largest portfolio of intellectual properties on MTT has offered research and commercial licences to several organisations. It has implemented the standard operation procedures for production of customised myoblasts for HCT, where approximately five grams of thigh muscle are obtained under local anaesthesia from heart patients between 40 and 90 years old. From this biopsy, about one billion purified myoblasts are grown in a Class 10,000 laboratory in about 30 days.

Cell manufacture follows cGMP. The cells are 99% pure, 95% viable and are tested by a third party to ensure that they are free of mycoplasma, bacteria, fungi and endotoxins. These cells will then be delivered with 20 injections placed 1cm apart between the viable and infarcted areas. Injections can be made through catheters or under direct visualisation in an open-heart bypass surgery.

Autograft Versus Allograft

Like muscular dystrophy, genetically defective hearts will need myoblasts from foreign normal

donors. Such allografts will be necessary for treating heart patients with infectious diseases to avoid contamination of the sterile culture laboratories. Allografts utilise well-characterised myoblasts that are readily available, allowing HCT to occur within 12 hours of myocardial infarction and potentially eliminating scarring upon regeneration. Scarring is unavoidable in an autograft, in which the patient's own myoblasts take three to four weeks to grow.

Whether autografts or allografts, HCT is the most logical alternative in prevention and treatment of heart conditions due to congestion, ageing, heredity or trauma. It may also be used to enhance heart contractility in anti-ageing.

Myoblasts – Not Stem Cells

Today, the field of cell therapy is confused and perplexed by the controversial stem-cell research, whereas gene therapy research is tainted by the mishaps of 'viral vector' technology. MTT does not involve the use of controversial stem cells or the use of dangerous viral agents that have been the cause of death in recent clinical trials. It has been proven safe on more than 230 human procedures in the past 12 years.

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Stem-cell technology has gained much attention due to the controversy of utilising cells from human embryos. More critically, scientists do not know the specific factor(s) that trigger stem cells to develop along a specific lineage, e.g., to differentiate only into heart muscle cells and not into other cell types. Such knowledge is not likely to be available within the next 10 years. Until then, stem-cell transplants into the heart may result in bony, cartilageous, fatty and fibrotic elements that are detrimental to heart function. Unlike the pluripotent stem cells, myoblasts are differentiated cells destined to become muscle.

Accordingly, muscle biopsy will be taken from the genetically ill patient. Myoblasts are cultured, purified and transduced before being injected intramuscularly into the patient. A substantial amount of the therapeutic gene can be harboured because the skeletal muscles constitute more than 50% of the volume of the body. Even a low liposome transduction efficiency with minimal therapeutic protein production can be compensated with a large quantity of myoblast transfer. The transduced muscle acts as a stable bioreactor to deliver a long-term supply of therapeutic protein at basal level.

Cell culture is the only way to generate new, live cells that are capable of surviving, developing and functioning in the body after transplantation, thereby replacing degenerated cells that are lost.

Future HCT

Myoblasts can be transduced to secrete vascular endothelial growth factor or various angiogenic factors to promote survival, development and functioning after HCT. In addition, the use of controlled cell-fusion technologies enables cell fusion between myoblasts and cardiomyocytes, producing heterokaryotic cardiomyocytes that are capable of extensive mitosis.

Cardiovascular disease is the number-one cause of death, and more than US\$280 billion is spent each year in an attempt to combat this. Heart muscle cells are terminally differentiated and do not divide significantly to regenerate the damaged heart muscle. When compared with a heart transplant, HCT eliminates the use of lifelong immuno-suppressants, which is the major cause of infection and death of heart transplant patients. HCT is much less invasive, and tissue availability is not an issue. At a fraction of the cost of a heart transplant, it also promises a reduction in health costs.

MTT Target Spectrum

Besides being in clinical trials with human neuromuscular diseases and myocardial infarction, animal studies have been published using MTT to treat diabetes mellitus (Type I and Type II), bone degeneration, cancer, anaemia, haemophilia, human growth hormone deficiency, muscle trauma, soft tissue augmentation, islet allograft rejection, pain and depression.³

Cell Therapy is Now

Good health is the wellbeing of all body cells. In hereditary degenerative diseases, sick cells need repairing and dead cells need replacing for maintenance of good health. Cell culture is the only way to generate new, live cells that are capable of surviving, developing and functioning in the body after transplantation, thereby replacing degenerated cells that are lost.

Myoblasts are the only somatic cells in the human body capable of natural cell fusion. The latter allows the transfer of all normal genes into genetically defective cells to effect phenotypic repair through complementation. MTT on Duchenne muscular dystrophy is the first human gene therapy demonstrated to be safe and effective over 12 years.

The use of MTT to transfer any other genes and their promoter/enhancers to treat other forms of diseases is under way. Myoblasts are efficient, safe and universal gene transfer vehicles, being endogenous to the body themselves. Since a foreign gene always exerts its effect on a cell, cell therapy will always be the common pathway to health.

Cell therapy is now. Given enough time for the FDA, the pharmaceutical companies and the medical community to understand its concept and implications, it will flourish to be the most important medicine in human history. After all, cells are what all life is made of. ■