IL-9 : 招請講演**Tissue Engineering to Rebuild the Lower Urinary Tract**

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We have tested the feasibility of muscle based gene therapy and tissue engineering for urological dysfunction using primary muscle derived cells (MDC) isolated by the preplate technique, that display stem cell characteristics. We then explored the potential use of these cells as an alternative injection agent for the treatment of stress urinary incontinence and impaired detrusor contractility.

Purified MDC were transduced with adenovirus or retrovirus carrying the gene encoding β -galactosidase and injected into the proximal urethral wall and the right and left lateral walls of the bladder of SCID mice. The tissues were harvested on days 5,15,35,70 and 6 months and assayed for β -galactosidase activity and the presence of innervated transduced myofibers was determined by acetylcholine receptors (AChR) staining to identify the location of neuromuscular junctions.

We observed a large number of transduced myotubes and myofibers expressing β -galactosidase at days 5,15,35,70 and 6 months post-injection. AChR staining showed the transduced myofibers were innervated into the injected bladder as early as 15 days post-injection. More importantly, we demonstrated that the injected MDC are capable of improving the contractility of the bladder and urethral muscle following an induced injury. Finally, we observed that some of the injected cells expressed α -smooth muscle actin at 5,35 and 70 days post-injection, suggesting their differentiation into smooth muscle.

In my presentation I will tell the story of how we got started with tissue engineering research in Pittsburgh and highlight some of our key findings and future plans. In the following section, I like to give two examples of ongoing research.

In summary, we have validated the feasibility of primary MDC transplantation into the detrusor or urethral wall as a potential treatment for stress urinary incontinence and impaired detrusor contractility. We have initiated clinical trial and our first patient was successfully treated in November 2004 at the University of Toronto.

Reducing Incontinence through Stem Cell Engineering

Stress incontinence is a problem that affects 13 million Americans. This occurs when the urethral sphincter muscles are weakened, as a result of pregnancy, aging, and in men, prostate surgery. The weak muscles cannot contract to prevent urine leakage.

Though there are various medical and surgical treatments that can help—such as collagen injections and pubovaginal sling surgery—Dr. Chancellor's research takes a new approach: asking the body to heal itself.

"We envision harvesting muscle stem cells from the adult patient, usually through a biopsy of the small muscles of the arm or leg," said Dr. Chancellor. "Stem cells have the ability to repair damaged muscles. So rather than just bulking up a deficient sphincter with collagen injection or creating a supporting weak urethra with a sling, we want to actually reengineer the deficient sphincter with muscle cells. We want to heal the deficient sphincter."

Dr. Chancellor emphasized that this procedure does not involve stem cells from any fetal or umbilical cord tissue. A small muscle biopsy can supply an adequate number of adult stem cells. Researchers have established a commercial bio-tech facility to culture the sample—and produce, over a few weeks, the millions of muscle-derived cells that will be used in clinical trials.

These cells would then be purified and injected into the patient's sphincter, causing it to rebuild muscle tissue and rejuvenate itself. The stronger muscle would hopefully regain function and control leakage.

In using this technique on rats, Drs. Chancellor, Yoshimura and de Miguel have improved the animal sphincter contractility by 88 percent. "There is minimal risk because the patient will be getting his or her own cells, and the procedure is minimally-invasive."

As with any research, progress is affected by limited resources. Regardless of whether the research involves

chemical processes, animal experiments or human clinical trials, all require skilled doctors and scientists, adequate laboratory space, sophisticated equipment and most importantly the money to pay for it. "To do good translational research for a practicing urologist is not easy" said Dr.Chancellor, "You have to wear many hats in a single day and there are obligations and pressures from all side." But it is at the same time exciting and I love waking up early in the morning thinking how to model a clinical problem in the research lab and try to come up a new treatment for a refractory clinical problem".

UPMC Researcher Seeks to End Post-Surgical Impotency

Some 179,000 American men will learn they have prostate cancer this year.Because of new medical and surgical treatments, the majority will survive, but many will be left with a degree of impotency, unable to resume normal sexual relations.

"Erectile dysfunction (ED) is common after any treatment for prostate cancer," said Dr.Chancellor, at the University of Pittsburgh Medical Center (UPMC), "but with nerve-sparing surgical techniques, for many men the dysfunction is temporary". However, most urologists know that some men, when faced with the diagnosis of prostate cancer, often turn away from radical prostatectomy, not that they are afraid of surgery, but they choose an alternative or refuse treatment out of fear of the potential complications of ED and stress urinary incontinence.

Surgical removal of a cancerous prostate gland, radical prostatectomy by open, laparoscopic or robotic technique, may damage the nerves controlling erections.Even with nerve sparing procedures, local inflammatory response may adversely effect the innervation of the penis and sphincter.The patient may be unable to achieve and maintain an erection.Obviously, this can affect his emotional health, relationships, marriage and self-esteem.

"Men are worry about ED after prostatectomy, and a man's fear of losing his potency may keep him from getting annual DRE and PSA check in the first place," Dr.Chancellor added.While Viagra has brought ED out of the closet, the little blue pill is not effective for everyone and long-term alternative treatment is certainly needed.

At UPMC's Center for Urology Research Excellence-ED, (CURE-ED) laboratory, however, Dr.Chancellor in collaboration with Naoki Yoshimura (MD, PhD) and Fernando de Miguel (PhD) are working on research that may reduce cases of ED by spurring new growth within the damaged nerves, and possibly speed the recovery of sexual functioning after surgery.

In experiments on male rats, they have created models of ED after prostatectomy by freezing, crushing and cutting the cavernous nerves, inducing a state of impotence.The CURE-ED team then used gene therapy to produce nerve growth factors, known as neurotrophic proteins, into the area around the cavernous nerves.The proteins are carried to the nerve by a specially constructed and nonreplicating herpes simplex virus vector. "Because herpes virus is preferentially taken up by nerves, it was the ideal vector to carry these proteins to help protect the cavernous nerves," said Dr.Yoshimura.

After nerve injury, researchers compared the pressure of the erection in rats receiving the regenerative proteins to those animals which received sham virus injection.The nerve growth factors led to a more rapid return of erection and increased intracorporal pressure.In addition, Dr.de Miguel, a molecular biologist demonstrated increased production of NOS and increased NOS nerve fibers in the ganglions and penile nerve fibers that contributes to erectile function.

The CURE-ED team has regulatory hurdles to overcome before the procedure can be tested on humans, particularly because the herpes virus is involved, but he foresees a time when gene therapy may be used before or at the time of a radical prostatectomy, and forestall or prevent ED before it begins.