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Stem cells and regenerative medicine in urology, part 1: General concepts, kidney, testis and urinary incontinence

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A B S T R A C T

Introduction: Progress in stem cell study and tissue engineering achieved in recent years proves that this may be one of the most promising research fields in the future. Most urological diseases could profit from the development of disciplines such as regenerative medicine as, up to now, there have been encouraging results in this subject.
Material and methods: We performed an electronic research through the Pubmed database, of both original and review publications, with the following search criteria: "stem cells urology", "kidney stem cells", "testis stem cells", "urinary sphincter", "cell therapy urology", "tissue engeneering urology" and "regenerative medicine urology".
Results: We reviewed 33 articles published up to January 2010, trying to summarize the most relevant findings in recent years, the clinical applications and the point we are at today.
Conclusion: Cell therapy and regenerative medicine are showing themselves to be one of the most promising fields within urological basic investigation in the last years. However, there is much work to be done yet, to make the advances reached in basic research applicable to the clinic.

Células madre y medicina regenerativa en urología, 1.ª parte: generalidades, riñón, teste e incontinencia

Palabras clave:
Células madre
Terapia celular
Medicina regenerativa
Ingeniería tisular
Urología

R E S U M E N

Introducción: Los progresos alcanzados en los últimos años en el estudio de las células madre y la ingeniería tisular demuestran que probablemente se trate de unos de los campos de la investigación más prometedores para el futuro. Gran parte de las enfermedades urológicas podrían beneficiarse del desarrollo de disciplinas como la medicina regenerativa, pues en la actualidad ya se han conseguido resultados esperanzadores en esta materia.

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Riñón
Testículo
Incontinencia urinaria

Material y métodos: Realizamos una búsqueda electrónica mediante la base de datos Pubmed, tanto de artículos originales como de revisiones, utilizando los criterios de búsqueda «stem cells urology», «kidney stem cells», «testis stem cells», «urinary sphincter», «cell therapy urology», «tissue engeneering urology» y «regenerative medicine urology».

Resultados: Hemos revisado un total de 33 trabajos publicados hasta enero de 2010, intentando resumir los hallazgos más relevantes de los últimos años y su aplicación clínica, así como el punto en el que nos encontramos hoy en día.

Conclusión: La terapia celular y la medicina regenerativa se están imponiendo como uno de los campos de investigación urológica más en auge en los últimos años. Sin embargo, queda aún mucho por investigar para que todos los rápidos avances de la investigación básica puedan ser trasladados a la clínica.

Introduction

Regenerative medicine and cell therapy are based on what is known about stem cells and their potential uses to regenerate damaged organs or tissues; this may offer effective treatments for conditions for which there are so far no satisfactory therapies. In recent years, knowledge in this field has experienced enormous growth, and many groups have devoted their work to opening new lines of research; thus, the field is increasingly important in all specialties. For various reasons, some specialties have taken the lead in this issue; urologists, however, have encountered a number of difficulties that have hindered to a certain extent the progress of research; these problems are, for instance, the fact that human urothelial, renal, and prostatic stem cells have not yet been identified and isolated, as well as the anatomical and physiological complexity of urologic organs like the kidney or the testicle. However, in the past few months we have seen great progress in regenerative medicine applied to urology, and the delay is being overcome. Today, the study of stem cells is one of the key research subjects in our discipline, not only in terms of regenerative medicine, but also because a better knowledge of those cells can clarify many questions about the cellular and molecular processes of carcinogenesis, which tend to be more closely related to tissue progenitor cells. The objective of this review is to summarize the general concepts of regenerative medicine, cell therapy, and tissue engineering, and to compile the most relevant findings and the latest studies published about kidney, testicle and urethral sphincter in order to learn about the advances in this field from the early days till today.

Material and methods

We used the Pubmed database to conduct an electronic search of original and review articles with the following criteria: “stem cells urology”, “kidney stem cells”, “testis stem cells”, “urinary sphincter”, “cell therapy urology”, “tissue engineering urology”, and “regenerative medicine urology”. We reviewed 33 studies published up to January 2010 about general concepts of cell therapy and regenerative medicine, and articles focusing on the urethral sphincter, the kidney, and the testicle. We excluded studies with inadequate methodologies or that did not deal directly with regenerative medicine, cell therapy, or tissue engineering in urology.

Stem cells: General concepts

Stem cells are defined by two fundamental features: the ability to multiply almost indefinitely, and the ability to generate mature cell lines. In contrast to the mature cells of adult organisms, stem cells are highly plastic, i.e., they can generate differentiated cells, in some cases even beyond the limits of the tissue in which they are found. The capability of one cell to differentiate into different cell types is known as potency. Totipotent stem cells are zygote cells; they can generate embryonic and extraembryonic tissues (germ cells, placenta). Pluripotent cells are the descendants of the above; they were initially described in the embryo four to six days old up to the blastocyst stage; they can originate the three germ layers and therefore all the body tissues derived from these layers, including gonadal tissue. Multipotent stem cells have been isolated from several tissues in the adult organism. Until recently, it was believed that they could only produce cells of a closely related cell strain; however, in the past few years numerous studies have been published that suggest the possibility of, for instance, transdifferentiation of multipotent mesenchymal stem cells of adipose tissue, umbilical cord or bone marrow into cells with mesodermic[1], ectodermic (neural)[2], and endodermic phenotypes, including pancreatic islet stem cells into mesenchymal phenotype cells, and bone marrow

or hair follicle stem cells into urothelial cells[3,4]. Recently, a study conducted by the researchers Fanyi Zeng et al from the Institute of Medical Genetics of Shanghai Jiao Tong University published in Nature, produced 37 iPS (induced pluripotent stem cells) lines from mouse fibroblasts (MEF [mouse embryonic fibroblasts] lines) with Yamanaka's four-factor transformation method. Using this technique with the iPS cell lines, the researchers produced 27 live mice entirely derived from iPS cells, whose first generation is now more than 9 months old. The authors report that they have also produced more than 200 second-generation iPS mice and 100 third-generation mice, which seem normal and healthy[5].

Embryonic and adult stem cells

There are two classic sources of stem cells. Embryonic stem cells are usually obtained through the disintegration of an embryo at the morula to blastocyst stage (however, due to the ethical conflicts associated with this, techniques to harvest stem cells without destroying the embryo have been developed, such as one recently developed at the Centro de Investigaciones Príncipe Felipe of Valencia with the human cell line VAL-10B, harvested from a single blastomere while maintaining the viability of the embryo[6]). Therefore, in most cases these are totipotent cells. Adult stem cells are undifferentiated, multipotent cells (although some are now being considered pluripotent) isolated from an adult tissue, placenta, or umbilical cord. In the adult organism there are pluripotent cells, albeit in a much smaller number than multipotent cells. For example, in bone marrow, one in every 10,000 cells is multipotent, while only one in every 1,000,000 is pluripotent.

Regenerative medicine and cell therapy

Regenerative medicine is based on what is known about stem cells and their potential uses to repair, regenerate, or replace damaged organs or tissues and thus treat diseases that to date are considered incurable; this is achieved through the employment of tissue engineering and cell, tissue, or organ transplantation. The first example of cell therapy is the transplantation of bone marrow progenitor cells to treat hematologic diseases such as bone marrow aplasia and leukemia. Furthermore, the potential clinical use of cell therapy is being researched for numerous diseases including diabetes mellitus, neurologic diseases such as Parkinson's, Alzheimer's and multiple sclerosis, for the regeneration of myocardial tissue after a heart attack, or the chronic fistulas of inflammatory bowel disease[7], urinary incontinence[8], various tumors, etc. To date, human bone marrow and fat have emerged as the most productive sources of adult stem cells; the difference is that the latter provides an access that is simpler and less aggressive to the patient. In principle, cell therapy has focused on the transplantation of autologous cells in order to avoid rejection; however, we know that maximum purification of adult stem cells leads to the same outcomes even if they are heterologous, as is the case of bone marrow transplantation.

Brief chronology

1963: The presence of stem cells with self-regenerative capability is shown in the bone marrow of mice.

1968: First successful bone marrow transplant between two siblings to treat severe combined immunodeficiency.

1978: Discovery of hematopoietic stem cells in human umbilical cord blood.

1981: Isolation of mouse embryonic stem cells.

1984: First stem cell cultures from teratocarcinomas.

1985: Development of a bioartificial skin on a bidimensional collagen matrix.

1987-8: First successful cultures of adult stem cells.

1992: In vitro culture of neural stem cells as neurospheres.

1997: First direct evidence of the existence of carcinogenic stem cells upon demonstration that hematopoietic stem cells may be the origin of leukemia.

1998: First human embryonic stem cell line. First germ cell cultures.

2000s: Multiple publications about the plasticity of adult stem cells, and clinical trials on cell therapy with this type of cells.

Regenerative medicine in urology

Urology is somewhat behind other specialties in terms of stem cell research. For example, while the characteristics and location of stem cells of other organs and tissues have been well known for years[9] and those cells have been cultured, expanded and even differentiated[10], the isolation and morphologic and biochemical characterization of kidney, prostate, and urinary tract stem cells have not been possible yet[11-13]. In the case of the urothelium, this may be due to the fact that it is a poorly understood epithelium: very few biochemical markers of differentiation have been found, the growth regulation mechanisms are not known, etc. In the case of the testicle, the major difficulty is finding functional germ cells capable of meiosis and gamete production; this is also due in part to the importance of the influence of the surrounding medium on differentiation. We know that the kidney has a very complex structure with 26 types of terminally differentiated cell types derived from four cell types originating from the intermediate mesoderm during embryonic development; they are arranged in the vascular, interstitial, glomerular, and tubular compartments[14]. The recently demonstrated existence of renal epithelial progenitors may explain the regression that occurs in some renal lesions[13].

Good outcomes have been obtained with embryonic stem cells, and even differentiation has been achieved; for example Oottamasathien's group in Nashville obtained urothelial-looking cells from mouse embryonic stem cells[15]; Kerkis's group was able to differentiate mouse embryonic cells into sperm cells and oocytes[16]. However, due to the ethical difficulties involved in attempting to apply this process to human beings, scientists are increasingly focusing their efforts in the study of adult stem cells, and this is being

very fruitful. Thus, a study published recently by a North American group shows that it is possible to transdifferentiate human amniotic fluid-derived stem cells (mesenchymal strain) into embryonic kidney epithelial cells by injecting them into mouse embryos[17]; this process takes advantage of the pluripotent character of the cells (similar to that of embryonic cells), and avoids the ethical and legal barriers of using the latter.

Sphincter tissue: Stem cells for treating urinary incontinence

Gregory S. Jack's group injected marked mesenchymal stem cells isolated from adipose tissue of women undergoing liposuction into the urethra and bladder wall of eight athymic rats and in the bladder wall of six mice. As a control, they used a group of eight rats injected only with culture-grade saline solution; they verified that the cells spread throughout the tissue, acquired morphological and biochemical characteristics of smooth muscle, and survived in the tissue beyond 12 weeks[18] (fig. 1).

In August 2007, Strasser's group at the University of Innsbruck published a study comparing the efficacy of intraurethral injection of mesenchymal stem cells (fibroblasts and myoblasts) vs. collagen. Twelve months after an ultrasonography-guided submucosal injection of fibroblasts and myoblasts in the striated sphincter, statistically significant results were obtained for all the parameters analyzed (quality of life, incontinence score, thickness of the striated sphincter, etc.) compared to those obtained after cystoscopy-guided intraurethral injection of collagen[19]. Several studies with similar outcomes have been published subsequently[20].

An Iranian group presented a study with children suffering from bladder exstrophy and poor response to bladder reconstruction surgery; the subjects underwent a muscle biopsy, and the cultured cells were intraurethrally injected; soon after, great improvement in the patients' incontinence and bladder capacity was observed[21].

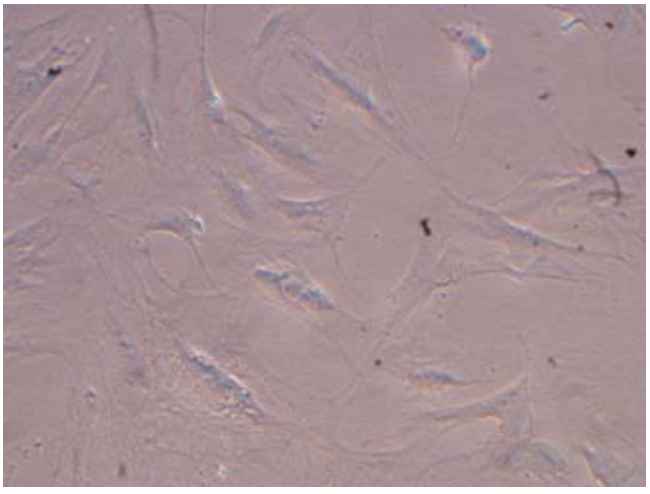

Figure 1 – Mesenchymal stem cells derived from cultured lipoaspirate.

Renal tissue: Structures capable of excreting a urine-like fluid

Bovine skin fibroblasts from steers were obtained and injected into the perivitelline space of enucleated oocytes (by nuclear transfer); the resulting blastocysts were implanted in female recipients previously prepared for implantation. At week 12, the resulting renal cells were cultured and expanded *in vitro*. Scaffolds were constructed consisting of three collagen-coated cylindrical polycarbonate membranes whose ends were connected to catheters terminating in a collecting reservoir. These scaffolds were populated with the cells obtained, and transplanted subcutaneously into the same animals. After 12 weeks, the scaffolds were removed and a biochemical analysis of the collected fluid, and a histological examination of the tissues were done. The biochemical tests revealed that the tissue had filtration, reabsorption, and secretory capabilities. The histological examination revealed a well-organized and vascularized tissue with glomeruli and tubules that showed continuity with the polycarbonate membrane. Immunohistochemical and polymerase chain reaction (PCR) tests were positive for proteins and renal cell-specific RNA[22,23].

Gonadal tissue: Infertility treatment

In 1994, a group from the University of Pennsylvania headed by Ralph Brinster first described a successful mouse testis stem cell transplantation. After isolating and injecting those cells into the seminiferous tubules of recipient mice (mutant strain lacking spermatogenesis), they found mature spermatozoa in the recipients[24].

Great advances in this field have been achieved since then, and there is a growing number of groups obtaining promising outcomes[25]. Most work at the experimental level has been done with rodents. In 2002, Van Pelt established cell lines with rat spermatogonia[26]. In 2006, Nayernia's group in Göttingen transdifferentiated mouse embryonic stem cells into spermatogonia that produced functional gametes. When they were transferred to oocytes by intracytoplasmic injection, pregnancies were achieved and taken to term, producing healthy mice[27]. Many groups are endeavoring to perfect these techniques described initially for culturing rodent germ stem cells[28,29]. Other groups, such as Lue's and Yazawa's, have attempted the transdifferentiation of bone marrow stem cells into germ cells. Eight busulfan-treated mice (busulfan is a chemotherapeutic that suppresses spermatogenesis and induces infertility) and eight c-kit mutant homozygous mice (which produce no germ cells) were the recipients; bone marrow stem cells from green fluorescent protein-producing mice were injected into the testicular interstitium and seminiferous tubules of the recipients. Ten to 12 weeks later, under fluorescent microscope, stem cells were visualized (thanks to the green fluorescent protein) surviving in the testicles of the recipient mice; some of the cells inside the seminiferous tubules had a Sertoli cell appearance (and even expressed FSH receptor). Live cells expressing P450scc (Leydig

cell marker) were found in the interstitium. Cells with the appearance of spermatogonia or spermatocytes expressing VASA (germ cell marker) were found only in the seminiferous tubules of busulfan-treated mice, but not in the c-kit mice. In any case, the concentration of these cells in the testes of busulfan-treated mice was much higher than in the other mice[30,31] (fig. 2).

In 2008, Skutella isolated and characterized germline stem cells derived from human testicle biopsies and found that they had features similar to those of embryonic stem cells. After transplantation into immunodeficient mice, a high rate of teratomas and a capability to differentiate into pluripotent cells derived from the three embryonic layers[32] were observed. This may provide a significant source of stem cells while avoiding the ethical dilemma of isolating embryonic cells. However, how to minimize the rate of teratomas is yet to be determined.

In our country, Carlos Simón's group in Valencia is also making great progress in the differentiation of human stem cells into germ cells[33].

Conclusion

Clinical research should not be limited to finding ways to stop the progression of certain diseases, but should also aspire to use these processes to repair damaged organs and systems and to fully recover their function. Cell therapy and regenerative medicine are thus becoming some of the most promising research fields in recent years.

The transplantation of kidneys produced by tissue engineering that avoids rejection and immunosuppressor treatment, or the production of spermatogonia from stem cells of different origins for the treatment of infertility are goals that seem utopic today, but may become a reality in the long term. However, there are still major technical hurdles to overcome, including finding matrices or culture media more adequate for the development of functional cells, as Brinster or Nayernia propose in the field of infertility,

In a promising field of stem cells, we believe that we urologists should join efforts to achieve results as soon as possible.

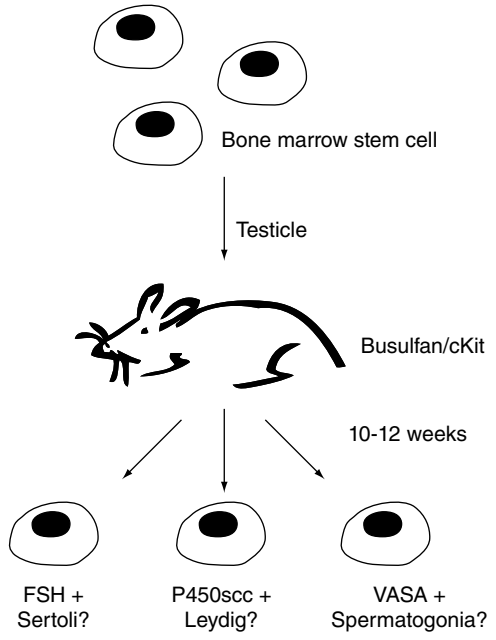

Figure 2 – Bone marrow stem cells injected into mice testes.

Conflict of interest

The authors state that they have no conflicts of interest.

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