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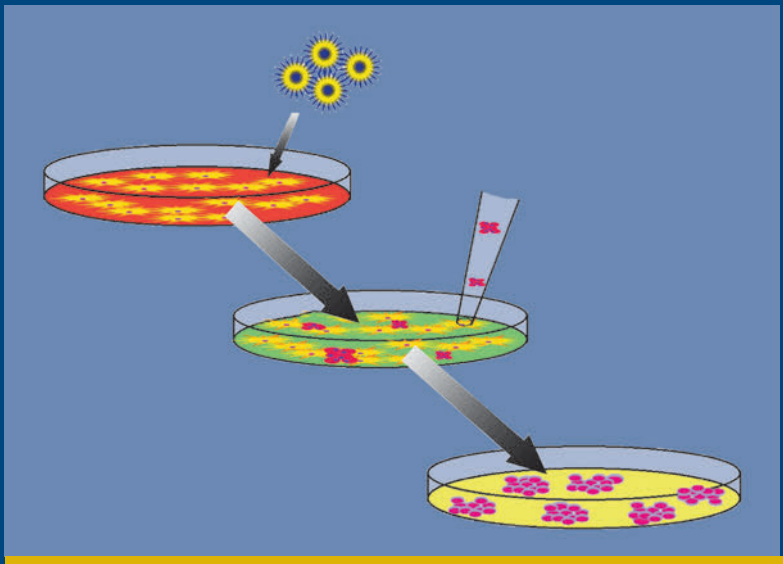
Berthold Seitz, Veit Flockerzi, Dieter Kohn, Markus Hoth,
Jens Rettig and Michael Böhm (Eds.)



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Einbandbild: Das Schema zeigt den Ablauf zur Generierung von induzierten pluripotenten Stammzellen. Titelbildgestaltung verändert nach Abbildung 2 im Beitrag Xu und Barry auf Seite 19 dieser Veröffentlichung. Dort findet sich auch die Beschreibung der einzelnen Verfahrensschritte.

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Contents

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Genetic Modification of Human Mesenchymal Stem Cells for Regenerative Approaches in Orthopedic Surgery

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(Homburg/Saar)

Dedicated to the memory of Kurt Mothes.

Abstract

Articular cartilage enables articulating joint surfaces to glide smoothly but has a limited ability to self-heal due to the lack of vascularization that can provide chondroregenerative cells at the site of injury. Cartilage that is damaged as a result of trauma or osteoarthritis (OA) does not properly restore its original hyaline structure (proteoglycans, type-II collagen) and mechanical integrity despite the availability of a number of clinical options. Instead, the repair tissue is of a mainly fibrocartilaginous nature (type-I collagen). It does not integrate well with the surrounding cartilage, is unable to withstand lasting stress and can lead to advanced stages of OA. Gene therapy is a powerful tool that enhances cartilage repair by delivering therapeutic (chondrogenic) gene(s) to the cartilage lesions. Among the various gene transfer vehicles, which have been manipulated to target both the cartilage and cells relevant to the reparative processes, highly effective and safe recombinant adeno-associated viral (rAAV) vectors have emerged alongside nonviral methods as preferred gene delivery systems in cartilage regenerative medicine. We report on the benefits of using such approaches to deliver various sequences (FGF-2, IGF-I, TGF-β, SOX9 as independent or combined treatments) at the site of injury. These approaches target progenitor cells *in vivo* and *in vitro*, either as isolated populations or as concentrates for implantation in cartilage lesions and as future tools for translational approaches in patients.

Zusammenfassung

Der Gelenkknorpel, der ein glattes Gleiten der Gelenkflächen der Gelenke ermöglicht, hat eine reduzierte Fähigkeit zur Selbstheilung, vor allem aufgrund eines Mangels an Vaskularisierung, die chondroregenerative Zellen an Orten von Knorpelverletzungen bereitstellen könnte. Das Problem der Knorpelreparatur besteht darin, dass durch Trauma oder Arthrose beschädigter Knorpel trotz Verfügbarkeit einer Reihe innovativer klinischer Optionen nie wieder identisch zu seiner ursprünglichen Struktur und mechanischen Integrität regeneriert werden kann. Stattdessen hat das Reparaturgewebe überwiegend eine faserknorplige Natur (Typ-I-Kollagen) und ist nicht optimal in den umgebenden Knorpel integriert. Der subchondrale Knochen ist verändert, und die so betroffene osteochondrale Einheit ist oftmals nicht in der Lage, dauerhaftem biomechanischem Stress zu widerstehen. Auf Basis des fokalen Defektes kann so eine Arthrose entstehen oder eine bereits bestehende Arthrose fortschreiten. Die Gentherapie ist ein potentes Instrument zur Verbesserung der Knorpelreparatur durch die Bereitstellung von therapeutischen chondrogenen Genen in Knorpelläsionen. Unter den verschiedenen Gentransfer-Vehikeln, die manipuliert werden, um sowohl den Knorpel als auch die Zellen, die für die Reparationsprozesse relevant sind, zu fördern, haben sich neben nichtviralen Methoden die hochwirksamen und sicheren rekombinanten Adeno-assoziierten viralen (rAAV) Vektoren als bevorzugte Genabgabesysteme in der regenerativen Knorpelmedizin etabliert. In diesem Beitrag wird über die Vorteile der Verwendung

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solcher Ansätze berichtetet, um verschiedene Gensequenzen (FGF-2, IGF-I, TGF-β, SOX9 sowohl als unabhängige oder kombinierte Therapien) an Stellen der Knorpelverletzung von Zielvorläuferzellen *in vivo* bereitzustellen und *in vitro* entweder als isolierte Populationen oder als Konzentrate zur Implantation in Knorpelläsionen und als zukünftige Werkzeuge für Translationsansätze bei Patienten zu verwenden.

1. Introduction

Articular cartilage is the tissue that covers the ends of bones in synovial joints. Its highly sophisticated structure permits articulating surfaces to glide smoothly. The cartilage chiefly consists of water, type-II collagen and glycosaminoglycans. Articular chondrocytes constitute the only cell type in this avascular and anural tissue. The articular cartilage is intimately connected to the subchondral bone plate, the most adjacent part of the subchondral bone. Defects in the articular cartilage pose a major clinical problem in orthopedic and trauma surgery. These defects are chiefly caused by osteoarthritis (OA) and trauma, but also originate from diseases affecting the subchondral bone, such as osteochondritis dissecans and osteonecrosis. Reconstructive surgical options aimed at repairing cartilage include the refixation of cartilage fragments, a variety of marrow stimulation techniques, high-tibial osteotomy, articular chondrocyte implantation, osteochondral transplants, and other approaches. Such techniques are primarily indicated for focal, non-OA cartilage defects. There are very few treatment options for the large and often ill-defined lesions caused by OA. All of the options aim to postpone the progression of the disease and to implant total joint replacements. Efforts to achieve regeneration of both types of lesions have been limited by the major challenge of stimulating resident and/or transplanted cells to form new, identical cartilage. When cell-based therapies, founded on the principles of tissue engineering, such as articular chondrocyte implantation, are employed, additional challenges arise with regard to obtaining transplantable chondrocytes (necessitating two individual operations) and to retaining these cells in the defect. Today this is accomplished by seeding them in a biodegradable matrix. Thus, even with the advent of such innovative techniques, the resulting repair tissue is always of a fibrocartilaginous nature, and articular cartilage regeneration never occurs in adults. Furthermore, there are special and temporal alterations of the subchondral bone in addition to the formation of the cartilaginous repair tissue. Their clinical significance, however, remains poorly understood.

2. Genetic Modification of Human Mesenchymal Stem Cells for Regenerative Approaches

The initial repair response to an articular cartilage defect is mediated, in part, by cell signaling polypeptides that act on cells derived either from the joint cavity or bone marrow. Chondrogenesis, the process of forming cartilage from mesenchymal progenitor cells involves key steps, such as mesenchymal proliferational condensation, chondroprogenital proliferation and differentiation, chondrogenesis, and terminal differentiation. It is tightly controlled by a variety of growth and transcription factors which regulate the production of key cartilage matrix proteins such as type-II, type-IX and type-XI collagen. Such factors influence the rate of articular cartilage and subchondral bone repair. The earliest studies from 1980, reporting on the application of growth factors as therapeutic tools, focused on the application of a bo-

vine brain fraction with fibroblast growth factor activity to enhance cartilage repair *in vivo* (Jentzsch et al. 1980, Wellmitz et al. 1980). Since then, the field had seen tremendous growth, chiefly in the identification of optimal factors for chondrogenesis, such as transforming growth factor beta (TGF-β) (Goldring 2006). Most interestingly, growth factors can be delivered by bioresorbable scaffolds to enable a controlled and prolonged delivery *in vivo* (Rey-Rico et al. 2017, Madry 2014). However, the application of growth factors as therapeutic agents for articular cartilage disorders has been restricted by their relatively short intra-articular residence time. The basic fibroblast growth factor (FGF-2) protein, for example, has a very short plasma half-life and is rapidly cleared of synovial fluid (Shida 1996, Frisch et al. 2015a). This necessitates either high doses or continuous delivery via osmotic pumps to achieve the physiological effect of the protein.

Gene transfer, the technique used to introduce foreign gene sequences into target cells, is a promising avenue for overcoming this problem. For the clinical problem of focal cartilage defects not caused by OA, a large body of literature supports the transplantation of genetically modified articular chondrocytes, which results in an improved cartilaginous tissue formation both *ex vivo* in three-dimensional model systems and *in vivo* in small and large animals (Madry et al. 2001, 2002, 2003, 2005, Johnstone et al. 2013).

Tissue engineering combined with gene therapy is also a promising approach for promoting articular cartilage repair. Cartilage constructs engineered from chondrocytes that overexpress human insulin-like growth factor I (IGF-I) improved the repair of osteochondral defects in a rabbit model. It should be noted that mature IGF-I constructs led to significantly reduced degenerative changes in the articular cartilage adjacent to the defects. Hence, the combination of spatially defined overexpression of a human growth factor gene within a tissue-engineered construct following bioreactor cultivation resulted in enhanced articular cartilage repair as well as a reduction in adjacent OA changes (Madry et al. 2013). Such genetically enhanced tissue engineering provides a versatile tool for evaluating potential therapeutic genes *in vivo* and for improving our understanding of the development of the repair tissue within the articular cartilage defects. Insights gained by additional exploration using this model may lead to more effective treatment options for acute cartilage defects.

Stem cells are a key focus of marrow stimulation because they constitute the type of cell which undergoes chondrogenesis and forms a cartilaginous repair tissue. They originate from the bone marrow compartment and can differentiate into chondrocytes but also osteocytes and adipocytes as a result of their plasticity. Gene therapy is a powerful tool for enhancing cartilage repair by delivering therapeutic (chondrogenic) gene(s) to cartilage lesions (Cucchiarini and Madry 2005, Cucchiarini et al. 2012, Madry and Cucchiarini 2013, Cucchiarini and Madry 2014a, Cucchiarini et al. 2014, 2015, Frisch et al. 2015c, Madry and Cucchiarini 2016). Gene transfer into human mesenchymal stem cells (MSCs) represents a particularly promising tool that would both increase chondrogenesis and provide a cell population capable of enhancing regeneration within the context of OA. Among various gene transfer vehicles that have been manipulated to target MSCs, chondrocytes and other cells relevant to the reparative processes, highly effective and safe recombinant adeno-associated viral (rAAV) vectors (Cucchiarini et al. 2003) have emerged as preferred gene delivery systems in cartilage regenerative medicine (Cucchiarini and Madry 2005, Cucchiarini et al. 2012, Madry and Cucchiarini 2013, Cucchiarini and Madry 2014a, Cucchiarini et al. 2014, 2015, Frisch et al. 2015c, Madry and Cucchiarini 2016). In contrast to the highly immunogenic and toxic adenoviral and herpes simplex viral vectors and to the integrative

retro-/lentiviral vectors that have a risk of insertional mutagenesis, rAAV vectors are safe due to the complete removal of viral coding sequences in the recombinant genome and to their maintenance as stable episomes. These vectors are particularly suited to delivering foreign gene sequences into human mesenchymal stem cells, a cell population which is difficult to genetically modify. A variety of studies have shown the potential of rAAV-mediated gene transfer to enhance the chondrogenic potency of mesenchymal stem cells that are derived from human bone marrow and used in cartilage repair. For example, studies have reported on the benefits of using such constructs to deliver various sequences (FGF-2, IGF-I, TGF-β, SOX9 as independent or combined treatments) at injury sites in order to target progenitors cells *in vivo* (Cucchiarini et al. 2005, 2013, Cucchiarini and Madry 2014b) and *in vitro*, either as isolated populations (Cucchiarini et al. 2011, Venkatesan et al. 2012, Frisch et al. 2014a, b, Tao et al. 2016) or as concentrates (Frisch et al. 2015a, Rey-Rico et al. 2015a, Frisch et al. 2016a, b). These can be implanted into cartilage lesions or be used as future tools in translational approaches in patients.

Among these approaches, rAAV-mediated overexpression of IGF-I in human bone marrow MSCs enhanced the production of major components of the cartilaginous extracellular matrix, proteoglycans and type-II collagen. At the same time it increased the DNA content, which is a measure of cell number. Growth factor-specific effects also appeared when FGF-2 or TGF-β were applied. Similar results were obtained when bone marrow concentrates were used instead of isolated MSCs (Rey-Rico et al. 2015a, Frisch et al. 2015b, 2016c, d), an environment more propitious to cell commitment due to the presence of natural biochemical factors and other cell types that may provide trophic agents necessary for proper differentiation.

Combining gene therapy via rAAV and tissue engineering platforms may offer optimal systems for the application or targeting of MSCs in regenerative medicine. In this light, an innovative approach is based on the delivery of this class of vectors to their targets by controlled release from biocompatible materials and scaffolds such as hydrogels (self-assembling peptide hydrogels, polxamer- and poloxamine-based micelles, alginate/poloxamer composites) (Rey-Rico et al. 2015b, c, Díaz-Rodríguez et al. 2015, Rey-Rico et al. 2016) and solid scaffolds (polyurethane, poly(ε-caprolactone) (Venkatesan et al. 2015, 2016). This prolongs the safe expression of rAAV transgenes, especially in a normal environment, and circumvents the presence of anti-AAV antibodies in 96 % of the population, 32 % of which is neutralizing antibodies (Chirmule 1999).

3. Outlook

Taken together, articular cartilage repair can be improved in experimental settings with potent gene and protein-based approaches. Human mesenchymal stem cells are the focus of such techniques since they constitute a relatively ubiquitous cell source that plays a key role in articular cartilage repair and regeneration. Basic scientific data from three-dimensional *ex vivo* models and small and translational large animal models will allow these findings to be translated into clinical strategies in the future.

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150 Jahre Mendelsche Regeln: Vom Erbsenzählen zum Gen-Editieren

Gemeinsames Symposium der Österreichischen Akademie der Wissenschaften (ÖAW), der Deutschen Akademie der Naturforscher Leopoldina – Nationale Akademie der Wissenschaften, der Veterinärmedizinischen Universität Wien und der Gregor-Mendel-Gesellschaft Wien
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Im Jahr 2016 gedachte die Gemeinde der Biowissenschaftler der Veröffentlichung der grundlegenden Arbeit des Brünner Mönches Gregor Mendel (1822–1884) *Versuche über Pflanzen-Hybriden*, in der die Grundlagen für die moderne Vererbungsforschung gelegt wurden. Nach der Wiederentdeckung der Mendelschen Regeln und der entsprechenden Publikation 1900 entwickelte sich die Genetik als wichtiger Zweig der Biologie. Der Band behandelt sowohl die Quellen und Anregungen für Mendels wissenschaftliche Arbeit unter neuen Gesichtspunkten als auch den Ablauf und die Hintergründe der „Wiederentdeckung", deren Geschichte aufgrund neuer Dokumente um weitere Aspekte bereichert werden kann. Darüber hinaus zeigt der Band die Erfolge der auf Mendel fußenden Genetik in verschiedenen Bereichen wie Pflanzenzüchtung, Biopharmazie und Veterinärmedizin. Er verweist aber auch auf die Nicht-Mendelsche Genetik und zeigt weitere Themenfelder gegenwärtiger Forschungen in den Lebenswissenschaften im Kontext ihrer historischen Voraussetzungen auf.

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Cellular Strategies in Osteoarthritis: Therapy and Pathogenesis

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Abstract

There is abundant evidence that stem cells – or tissue progenitor cell populations – reside in many adult tissues and play a role in tissue homeostasis and repair. Their differentiation potential is more limited than embryonic stem cells; however, they remain an attractive option in the development of therapeutic strategies. Mesenchymal stromal cells (MSCs), isolated from bone marrow, adipose tissue and other sources, have received some attention as innovative and promising advanced therapeutics that can be used to treat a broad spectrum of conditions. These cells have been at the forefront of investigations in regenerative orthopedics for some time and may represent a new way of repairing cartilage and treating osteoarthritis. It is important to note that our understanding of the potential therapeutic mechanism(s) of action of MSCs has been evolving. Early efforts relied on a tissue engineering paradigm based on the use of cells to replace tissue. This has evolved more recently into the concept of paracrine activity. Thus, there has been a shift in our understanding of the biological nature and therapeutic activity of MSCs, from progenitor cell to immunomodulatory and anti-inflammatory cell.

In addition to MSCs as therapeutic cells, there is enormous potential and global interest in using induced pluripotent stem cells to study cellular pathology and phenotypes in a disease- or patient-specific manner. This is especially so in the case of osteoarthritis, a condition for which there are no regenerative or disease-modifying therapies.

Zusammenfassung

Es gibt viele wissenschaftliche Beweise, dass Stammzellen – bzw. Gewebe-Stammzell-Populationen – in vielen adulten Geweben vorkommen und bei der Gewebe-Homeostase und Reparatur eine Rolle spielen. Ihr Differenzierungspotential ist zwar eingeschränkter als bei embryonalen Stammzellen, dennoch bleiben sie eine attraktive Option bei der Entwicklung therapeutischer Strategien. Mesenchymale Stromazellen (MSCs), isoliert aus dem Knochenmark, aus Fettgewebe und anderen Quellen, haben als innovative und vielversprechende erweiterte Therapeutika viel Aufmerksamkeit gefunden, die für die Behandlung eines breiten Spektrums an Zuständen angewendet werden können. Diese Zellen stehen seit einiger Zeit an vorderster Front der Entwicklungen in der regenerativen Orthopädie und könnten ein neuer Weg zur Regeneration von Knorpel und zur Behandlung von Osteoarthritis sein. Es ist wichtig festzuhalten, dass sich unser Verständnis für den (bzw. die) potenziellen therapeutischen Wirkungsmechanismus(en) der MSCs weiterhin entwickelt. Frühere Versuche folgten einem *Tissue-engineering*-Paradigma, das auf der Verwendung von Zellen als Gewebeersatz basierte. Das hat sich in neuerer Zeit zu einem Konzept parakriner Aktivität entwickelt. Auf diese Weise gab es einen Wandel in unserem Verständnis der biologischen Natur und therapeutischen Aktivität von MSCs – von Vorläuferzellen hin zu immunmodulatorischen und entzündungshemmenden Zellen.

In Ergänzung zu den MSCs als therapeutischen Zellen gibt es ein enormes Potential und globales Interesse für die Anwendung induzierter pluripotenter Stammzellen, um zelluläre Pathologie und Phänotypen in krankheits- bzw. patientenspezifischer Weise zu untersuchen. Dies ist besonders bei der Osteoarthritis, einer Erkrankung, für die es keine regenerativen bzw. verlaufsverändernden Therapien gibt, der Fall.

1. Introduction

Osteoarthritis (OA) is a complex condition with a broad pathology characterized by damage to the articular cartilage and changes in the subchondral bone and synovium (de Lange-Bro-

KAAR et al. 2012, FINDLAY 2010, GOLDRING and GOLDRING 2010). Disease progression involves extensive degeneration of articular cartilage and morphological changes that include subchondral bone thickening, the development of osteophytes, and synovitis. The disease greatly impacts the patient's quality of life, and the economic costs associated with treatment and rehabilitation are profound. Despite this, no approved drug treatment, biological therapy or procedure prevents or delays the progressive tissue degeneration that occurs in the OA joint, and all of the interventions only modify symptoms. There is certainly a need for new regenerative treatment options and mesenchymal stromal cells (MSC) therapy may represent an attractive modality in this regard. This paper discusses progress and possibilities for new regenerative therapies in OA involving delivery of these cells.

A further development in the context of stem cells and disease has been the powerful and transformative discovery of induced pluripotent stem cells (iPSCs). This technology now makes it possible to generate embryonic-like stem cells by reprogramming adult cells such as dermal fibroblasts. This has resulted in exceptional new opportunities in disease modelling, high throughput screening, and an understanding of stem cell plasticity. The use of iPSC technology to study OA has been under consideration.

2. Osteoarthritis

Osteoarthritis is a degenerative joint disease where there is progressive loss of articular cartilage associated with increased water content and loss of proteoglycan from the extracellular matrix. In morphological terms, there is erosion of the cartilage surface with evidence of fibrillation. Changes in bone structure in OA include the formation of osteophytes and subchondral sclerosis (increased trabecular thickness and decreased numbers of trabeculae) as well as a more porous subchondral plate. Other tissues, including ligaments, tendons and synovium, are also involved.

An attempt was made with OA to initiate an apparent endogenous repair response associated with chondrocyte proliferation and an increase in proteoglycan synthesis. This was ultimately unsuccessful so that, with current approaches, OA is an irreversible degenerative disease. Indeed, it is striking that no approved drug treatment, biological therapy or procedure delays or reverses the disease, and only symptomatic treatment options are available. These include controlling pain using steroidal and nonsteroidal anti-inflammatory drugs, viscosupplementation, and the use of nutraceuticals such as chondroitin sulfate or glucosamine.

Arguably, several factors contribute to the lack of progress in the development of treatment options for OA. These include its complex pathology, lack of biomarkers, poor early diagnosis and a limited understanding of the disease mechanism. Previous attempts at developing new treatment modalities, such as those targeting interleukin-1 activity or matrix metalloproteinases, have met with limited success. It does appear that OA may be a mesenchymal disease in which the activity, phenotype or mobilization of MSC populations are altered, leading to an absence of repair and increased degenerative changes. As a result, it can be hypothesized that all of the tissues that comprise the healthy joint depend on the availability and activity of MSCs for proper development and homeostasis. In conclusion, the delivery of exogenously prepared MSCs could have a regenerative effect.

3. Mesenchymal Stromal Cells

Human MSCs (hMSCs) are isolated from tissue biopsies such as bone marrow harvested from the superior iliac crest of the pelvis. The cells represent a small fraction of the total nucleated cell population in marrow but are capable of efficient expansion in culture. hMSCs are cultured in basal media, often with fetal bovine serum, for 12–14 days. This allows for the selective expansion of the adherent MSCs which retain a fibroblastic morphology. As far as characterization of hMSCs is concerned, a consensus for laboratory-based and pre-clinical investigation has been proposed (Dominici et al. 2006). The consensus suggests that hMSCs must (*1*) adhere to tissue culture plastic, (*2*) show positive expression of CD105, CD73 and CD90, (*3*) show negative expression of CD34, CD45, HLA-DR, CD14 or CD11b, CD19 or CD79α, and (*4*) be capable of differentiation into osteoblasts, adipocytes and chondrocytes under standard conditions.

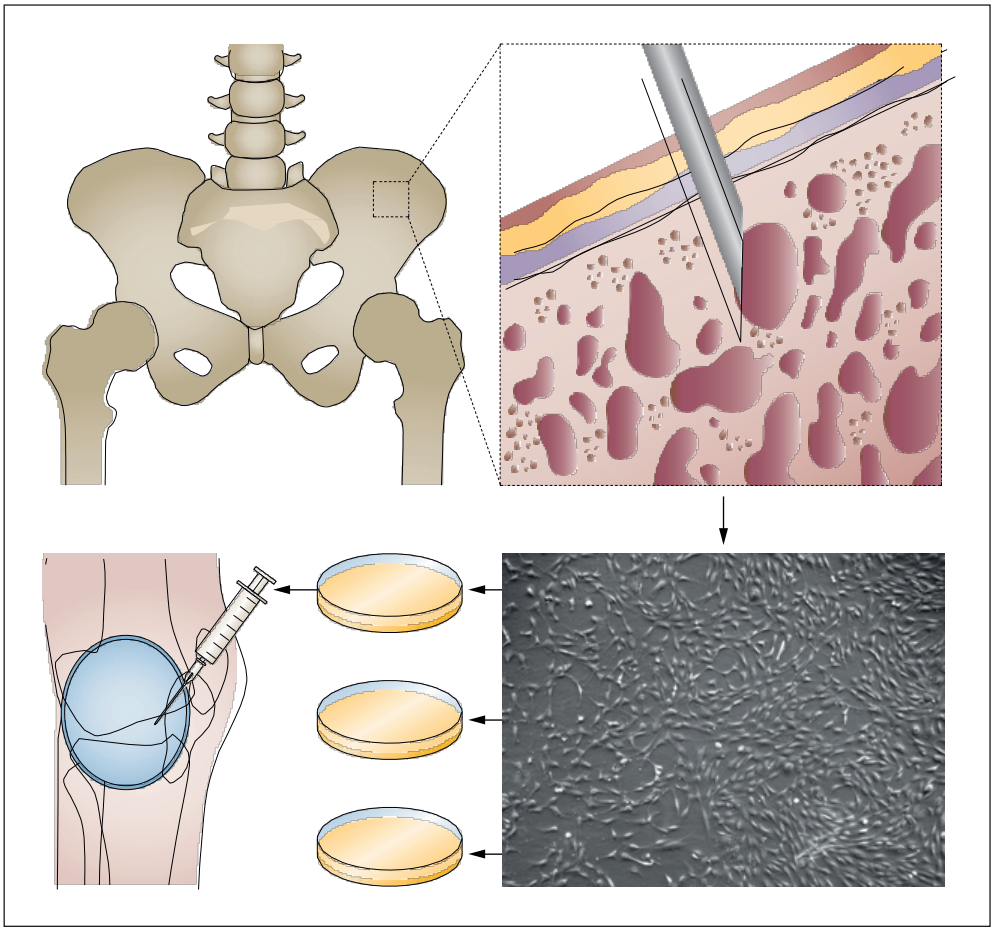

Fig. 1 Cell therapy for the treatment of osteoarthritis using bone marrow-derived MSCs. From Barry and Murphy 2013.

4. Mesenchymol Stromal Cell Therapy for Osteoarthritis

The effects of MSCs have been studied in a caprine model of traumatic OA (Little et al. 2010). Following intraarticular delivery of bone marrow-derived MSCs suspended in hyaluronan to meniscectomized joints, significant meniscal regeneration and chondroprotection were observed compared to joints that only received hyaluronan (Murphy et al. 2003). Using labelled MSCs it was apparent that the transplanted cells contributed minimally to repair and were detected only at the surface of the newly formed tissue. Although meniscal regeneration was accompanied by a marked repair response with cartilage protection and a reduction in osteophytes, the authors concluded that the implanted MSCs induced a host repair response by releasing paracrine factors rather than by directly contributing to tissue regeneration.

The effectiveness of intraarticular delivery of MSCs in the knee or stifle has also been tested in preclinical OA models in mice (Diekman et al. 2013), rabbits (Toghraie et al. 2012), rats (Horie et al. 2012), guinea pigs (Sato et al. 2012), sheep (Al Faqeh et al. 2012), and dogs (Guercio et al. 2012). In these models, treatment resulted in delayed OA progression. Furthermore, in a collagenase-induced OA in the mouse disease, progression was also modulated by the intraarticular injection of adiposederived MSCs, resulting in the protection of the articular cartilage and a reduction in synovial thickening (ter Huurne et al. 2012).

These impressive preclinical studies have given rise to several efforts in clinical testing. A recent meta-analysis (Cui et al. 2016) of 18 published trials suggested that MSC treatment significantly improved pain and functional status relative to basal evaluations in knee OA, and that this effect was sustained for two years following treatment. This was an important conclusion and is certainly an impetus for further study. Nevertheless, more work clearly needs to be done, and more detailed clinical evaluation is necessary to determine whether the effects are structural or symptomatic. Many of the earlier clinical studies addressed the use of MSCs to repair focal cartilage defects of the knee rather than OA, resulting in several positive observations. Wakitani et al. (2007) treated 12 patients with autologous bone marrow-derived MSCs which were delivered directly to the OA cartilage defect in a collagen gel. The cell-treated group showed some transient improvement which was not sustained after 6 months.

A study was conducted by Pers et al. (2016) who carried out a dose-escalation protocol of intra-articularly injected, autologous, adipose-derived stromal cells in patients with knee OA. Their aim was to assess safety as a primary, and clinical efficacy as a secondary, endpoint. Although small and uncontrolled, this study nonetheless showed that the procedure was safe and well-tolerated and also provided encouraging preliminary evidence of efficacy. Another randomized controlled study was recently published in which knee OA was treated with allogeneic bone marrow MSCs (Vega et al. 2015). The study suggested that cell-treated patients displayed significant improvement in functional outcome compared to control patients treated with hyaluronic acid. Furthermore, quantification of cartilage quality by T2 MRI relaxation measurements showed significant improvements in cartilage quality in the MSC-treated patients. The essential message highlighted by these results is the absolute need for larger and better controlled studies to provide unambiguous and definitive information on the effectiveness of this technology.

5. Induced Pluripotent Stem Cells

The extraordinary and transformative work of Takahashi and Yamanaka (2006) describes a method to convert adult fibroblasts to pluripotent stem cells by introducing four transcription factors. This has had profound implications for stem cell biology, regenerative medicine and understanding disease mechanisms. The fully pluripotent nature of these reprogrammed cells was demonstrated by their capacity to differentiate into cells representing all three embryonic germ layers and by their capacity to form teratomas *in vivo*. This discovery has led to new insights into the regulation of differentiation and has allowed greater understanding of the nature of stemness and fate decisions. One exceptionally important outcome of this has been the capacity to generate patient-specific iPS cells from dermal fibroblasts and to use these to provide new cellular models of disease pathways and new screening tools to test pharmacological and biotherapeutic interventions.

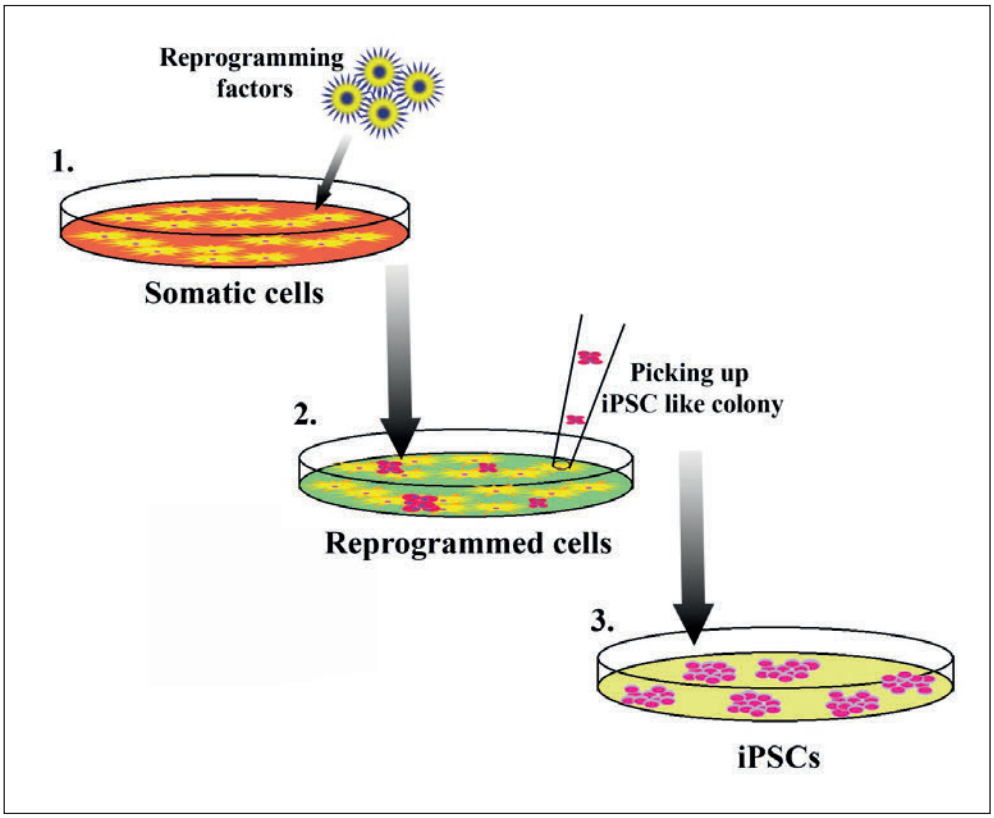

Fig. 2 Scheme depicting the steps for the generation of induced pluripotent stem (iPS) cells. (*1*) Reprogramming is initiated by insertion of reprogramming factors into somatic cells. (*2*) Transduced cells expressing exogenous reprogramming factors undergo mesenchymal-epithelial transition and iPSC-like colonies are manually selected. (*3*) Selected colonies, with characteristics similar to embryonic stem cells, are expanded in culture for establishing the iPSC line.

Kim et al. (2011) generated iPSCs from OA patient synoviocytes. They showed that the iPSCs possessed strong chondrogenic propensity, immediately providing an endless supply of cells for the study of the pathogenesis of OA. Diekman et al. (2012) generated chondrocytes from iPSCs derived from mouse fibroblasts and demonstrated integration in an *in vitro* model of cartilage repair. Borestrom et al. (2014) showed that iPSCs could be derived from chondrocytes which had the capacity to redifferentiate into cartilage matrix-producing cells *in vitro* in a manner that was superior to fibroblast-derived iPSCs. These studies represent new ways of providing large numbers of chondrocytes for autologous transplantation as well as new opportunities for studying OA pathogenesis.

One of the complicating factors in generating iPSCs from OA patients relates to the complex nature of the disease itself. OA is a broad-spectrum condition with a multiplicity of disease subtypes. It can affect one or many joints, and there is great variation in the severity of symptoms and in the degree of tissue damage. The pathogenesis of OA is poorly understood although multiple factors contribute to its development. These include the presence of acute injury in the ligaments, meniscus, cartilage and other tissues, obesity, exercise, lifestyle, and genetic factors. All of these factors make it more difficult to generate meaningful disease models, and the vast bulk of work on OA pathogenesis has relied on animal models involving surgically induced trauma. It does seem that a more easily interpretable model would arise if there was a single identifiable causative factor, such as a gene mutation. While this does not exist for idiopathic OA, there are related conditions where a clear genetic linkage does exist. One such monogenic disorder is familial osteochondritis dissecans (FOCD) characterized by multiple joint involvement, short stature and early onset OA (Andrew et al. 1981, Phillips and Grubb 1985, Stattin et al. 2008, Stougaard 1964). One type of FOCD displayed an autosomal dominant pattern of inheritance and was found to be associated with a heterozygous G-A mutation in exon 17 of the ACAN gene resulting in a Val-Met amino acid replacement (V2303M) in the G3 aggrecan C-type lectin domain (Stattin et al. 2008, 2010).

Xu et al. (2016) reported on the generation of iPSCs from dermal fibroblasts taken from patients with this inherited mutation. They also isolated MSCs from the marrow of symptomatic patients. The MSCs had a normal capacity to differentiate into osteocytes and adipocytes but the chondrogenic pellets were irregular, discoid and larger than those generated from control MSCs. This provided some strong insight into the cellular pathology of the condition and the consequences of the ACAN mutation. Detailed immunohistochemical analysis indicated that, in the patient chondrogenic pellets, synthesis of the aggrecan core protein was unimpaired but processing was defective. The striking observation was that newly synthesized aggrecan was trapped within the chondrocytes and was not released to the extracellular matrix (ECM). This resulted in endoplasmic reticulum (ER) stress and an upregulation of expression of ER stress-related proteins. The same observation was made when cartilage derived from patient iPSCs was studied. This indicates that the disease phenotype was preserved after induction of pluripotency.

A further striking observation was made by quantitation of a number of ECM proteins in chondrocytes differentiated from patient MSCs. This indicated that ECM assembly was globally disregulated, with marked upregulation of expression of some small leucine-rich proteoglycans (SLRPs). Of these, asporin (class 1 SLRP), osteoadherin (class 2 SLRP) and mimecan (class 3 SLRP) were dramatically upregulated in patient cells. These particular SLRPs have all been linked to active mineralization (Balakrishnan et al. 2014, Bonucci 2012), a particular feature often seen in OA (Ea et al. 2011). These observations provided

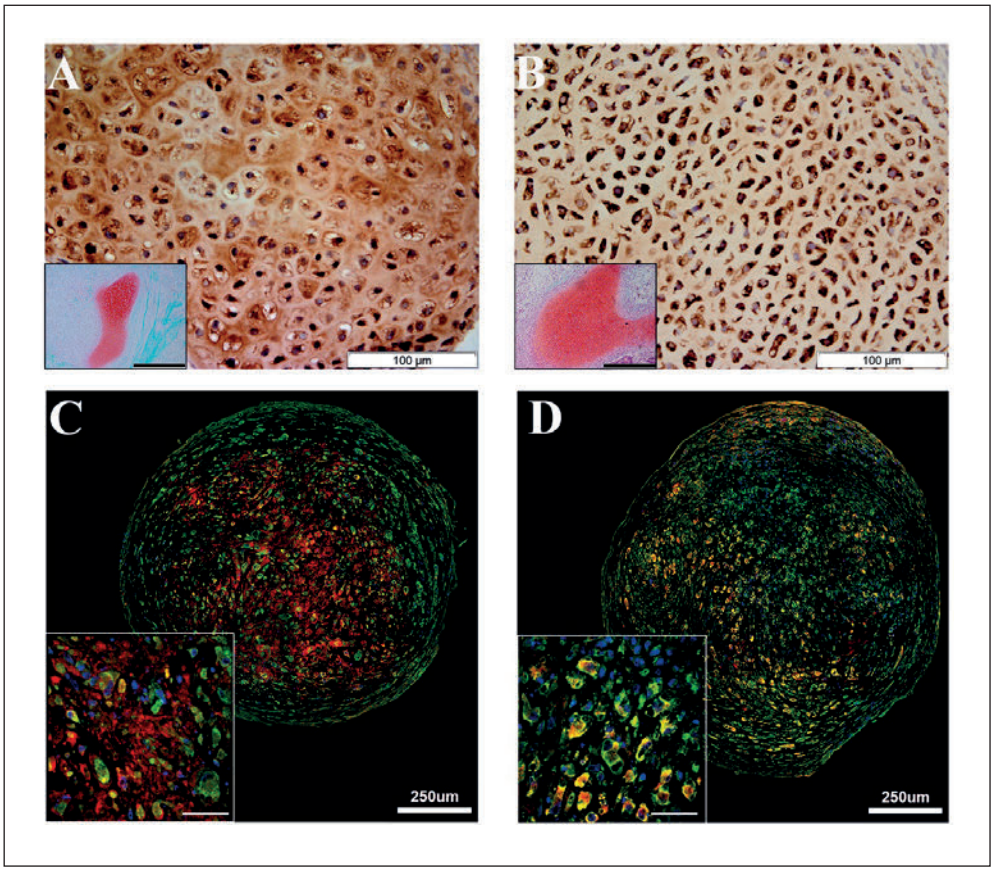

Fig. 3 Aggrecan intracellular accumulation in cartilaginous tissues differentiated from FOCD iPSCs, *in vivo* and FOCD BM-MSCs, *in vitro*. Representative images show cartilage in teratoma derived from control iPSCs (*A*) and FOCD iPSCs (*B*). Immunohistochemical staining was performed to assess the distribution of aggrecan protein (brown) in extracellular matrix (ECM) (scalar bar, 100 μm). Cell nuclei (blue) were stained by hematoxylin. The embedded images in (*A*) and (*B*) show the corresponded cartilage (red) form in teratoma, which was located by Safranin-O staining (black scalar bar, 500 μm). To evaluate the intracellular location of aggrecan protein, multiple immunofluorescent staining was applied on chondrogenic pellets derived from control BM-MSCs (*C*) and FOCD BM-MSCs (*D*). Aggrecan was stained as red. GRP78, a marker of endoplasmic reticulum was stained as green. The colocalization of these two proteins is shown as yellow (scalar bar, 250 μm). Insets of (*C*) and (*D*) are magnified images of the corresponded chondrogenic pellets (whit scalar bar, 50 μm). From Xu et al. 2016.

some understanding of ECM disregulation that gives rise to the symptoms of FOCD and the consequential development of OA. These include a poorly organized ECM that lacks aggrecan and has uncontrolled levels of other matrix components resulting in the formation of cartilage without an organized structure and mechanical integrity, the early appearance of cartilage defects (the canonical signs of OCD), pronounced susceptibility to OA, and the need for multiple early joint replacement surgeries. This study does not address the characteristic of short stature, also seen in these patients. However, it is easy to speculate that the emergence of hypertrophic chondrocytes and the formation of the growth plate are also impacted by the same ECM disassembly seen in the articular cartilage. This has not yet been studied.

6. Conclusion

OA is regarded as a major cause of disability worldwide and its prevalence is likely to increase as a result of demographic changes associated with aging populations, lifestyles and obesity. While joint replacement surgery has been remarkably successful and continues to be the main type of treatment for patients with advanced disease, there is nonetheless a very significant burden in terms of poor quality of life and high healthcare costs. No disease-modifying treatments are available today despite years of effort and multiple international research initiatives targeting new drugs, improved device technology, advanced biologic therapies and strategies for unraveling the underlying molecular pathogenesis of OA. The use of cellular therapy as a potential regenerative treatment for OA has to be carefully assessed. Early results from clinical trials are promising but none of these trials are conducted on a scale that makes them conclusive enough. Two aspects of cell technology require a great deal more effort: (*1*) the assessment of cell delivery as a potential new therapy and (*2*) the generation of cell-based models of OA to augment the work that has previously been carried out using animal models. In terms of cell delivery, MSCs may represent the most promising option but this requires clear confirmation in well-designed clinical trials that are large enough to achieve statistical significance. In terms of cell-based models, there is a need for a much greater effort to utilize iPSCs to study OA pathogenesis and to provide new screening tools. This will require the generation of large banks of iPSCs from patients with a broad spectrum of conditions. Through skillful application of these new technologies, the future may be brighter for patients with OA.

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The Allogenicity of Stem Cells and their Immunobiological Hurdle for Clinical Application

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Abstract

Stem cell transplantation is a rapidly developing field and an attractive therapeutic way to regenerate tissues injured by cardiovascular disease. However, researchers are still trying to find the best way to use stem cells in clinical treatment. This starts with the choice of the right type of stem cells. Autologous, patient-specific adult stem cells are currently under investigation. Because they are derived directly from the patient, they are not subject to immune rejection. However, their efficacy with regard to myocardial regeneration is still under debate. Pluripotent stem cells hold more promise for the treatment of heart failure since they are capable of differentiating into cardiomyocytes and building new cardiac tissue. But these cells are allogeneic and will get rejected after transplantation. Future application of allogeneic stem cells requires the engineering of ready-to-use stem cell lines that can be quickly and easily prepared and applied to a large pool of patients. This will require the availability of "off-the-shelf" cell products within a short, critical window of time that are widely immuno-compatible. The natural phenomenon of fetomaternal tolerance is a unique example of how allogeneic tissue can evade the "host's" immune system by inducing local tolerance. Here, we present the idea of generating a hypo-immunogenic stem cell line by adapting the features of fetomaternal tolerance. Stem cells will need to undergo modifications of different cell surface molecules.

Zusammenfassung

Die Stammzelltransplantation ist ein sich schnell entwickelndes Gebiet und eine attraktive therapeutische Option zur Regenerierung von durch eine kardiovaskuläre Erkrankung verletztem Gewebe. Dennoch versuchen Forscher noch immer, den besten Weg zur Nutzung von Stammzellen für klinische Behandlungen zu finden. Dies beginnt mit der Wahl des richtigen Stammzelltyps. Derzeit werden autologe, patientenspezifische adulte Stammzellen untersucht. Da sie direkt vom Patienten stammen, sind sie nicht der Immunabwehr ausgesetzt. Für die myokardiale Regeneration wird ihre Effizienz jedoch noch diskutiert. Pluripotente Stammzellen sind für die Behandlung von Herzinsuffizienz vielversprechender, da sie in der Lage sind, in Kardiomyozyten zu differenzieren und neues Herzgewebe aufzubauen. Diese Zellen sind jedoch nach einer Transplantation allogen und werden abgestoßen. Die zukünftige Anwendung allogener Stammzellen erfordert die Konstruktion von gebrauchsfertigen Stammzelllinien, die schnell und einfach vorbereitet und bei einem großen Pool von Patienten angewendet werden können. Zu diesem Zweck werden „Off-the-Shelf"-Zellprodukte in einem kurzen kritischen Zeitfenster benötigt, die weitgehend immunkompatibel sein müssen. Das natürliche Phänomen der fetomaternalen Toleranz ist ein einzigartiges Beispiel, wie allogenes Gewebe durch Induzierung lokaler Toleranz dem Immunsystem des Wirts ausweichen kann. In diesem Beitrag stellen wir das Generierungskonzept einer hypo-immunogenen Stammzelllinie durch Adaptierung der Merkmale der fetomaternalen Toleranz vor. Stammzellen müssen sich der Modifizierung verschiedener Zelloberflächenmoleküle unterziehen.

1. Introduction

Cardiovascular diseases are still the leading cause of death worldwide (Benjamin et al. 2017). After a heart attack, for example, the damaged tissue cannot be replaced or enhanced by the organism itself due to the heart's limited regenerative potential. Organ transplantation is limited by a scarcity of donors and is associated with a risk of rejection (Synnergren et al.

2012) and long-term immunosuppressive therapy. Therefore, new strategies have to be found in this area that provide a good and effective option for all patients.

Stem cell transplantation is a promising alternative to organ transplantation but immune rejection afterwards is also a potential problem with this type of therapy. Our laboratory has demonstrated in various animal models (Kolk et al. 2009) that ES cells are rejected after transplantation mainly as a result of T cell activation (Deuse et al. 2011, Swijnenburg et al. 2008a, b). We now aim to better understand the immunobiology of human ES cells and how to decrease their immunogenicity by learning from nature. During pregnancy, the fetus expresses paternally inherited allo-antigens but is nevertheless tolerated by the maternal immune system. This is a unique example of how the immune system modulates a destructive allo-immune response into a state of tolerance. We believe that this natural mechanism may have major implications in the development of novel strategies to induce immunologic tolerance in cell transplantation.

In this overview we introduce a new technique for reducing the immunogenicity of stem cells by generating hypo-immunogenic cells. The mechanisms of fetomaternal tolerance served as a model.

2. The Prospects of Stem Cell Therapy

The most urgent problem facing transplantation is the lack of donor organs. One possible alternative to organ transplantation is stem cell therapy, the aim of which is to replace, repair, or enhance the biological function of the diseased heart or damaged heart tissue. This requires differentiated cardiomyocytes from stem cells. Adult stem cells are multipotent which means that the differentiation can only occur within the germline.

Pluripotent stem cells, like embryonic stem cells or induced pluripotent stem cells, are more promising. The latter can be generated directly from adult somatic cells by introducing reprogramming factors like Oct4, Sox2, cMyc and Klf4 (Takahashi und Yamanaka 2006). Induced pluripotent stem cells have an embryonic stemness characteristic similar to embryonic stem cells, although epigenetic signatures of the parent cell may persist and affect its functionality over time.

Because pluripotent stem cells can be differentiated into several cell types, the potential application of stem cell therapy is wide-ranging. Differentiation can be performed *ex vivo*, allowing researchers to monitor the procedure and generate the proper population of cells prior to transplantation. Undifferentiated pluripotent stem cells need to be avoided due to their potential for teratoma formation.

In most cases, stem cell-derived derivates (e.g. cardiomyocytes) are used for cell therapies. The transplantation of stem cell-derived cardiomyocytes in various animal models has already had some functional success in regenerating ischemic heart tissue (Yang et al. 2002).

3. Different Types of Stem Cells – Which is the Best?

For the clinical application of stem cell therapy, three major types of pluripotent stem cells are considered: embryonic stem cell (ES cells), somatic cell nuclear transfer (SCNT)-based ES cells and induced pluripotent stem (iPS) cells. Designing more effective treatment options

requires knowledge of the pros and cons behind each of the stem cell types, as well as the immunological reasons behind post-transplant rejection of pluripotent stem cells.

3.1 The Immunogenicity of ES Cells

The initial idea of stem cell therapy began with the generation of the first human embryonic stem (ES) cell line. These pluripotent cells can be obtained from the blastocyst stage of embryonic development and have the ability to self-regenerate and differentiate into all of the different cell types. The generation of these ES cells is connected with ethical concerns, and their generation is tightly restricted. Nevertheless, ES cells have already been utilized in human pilot studies.

There were initial reports suggesting that ES cells are immune privileged (Drukker et al. 2002) based on their low levels of surface expression of major histocompatibility complex (MHC). We and other laboratories showed that ES cells are vigorously rejected after transplantation. Furthermore, the rejection speed increased upon repeat transplantation, suggesting that rejection was promoted by the adaptive immune system (Swijnenburg et al. 2008b). Although MHC expression levels are low, they seem to be enough to activate the immune system and trigger an immune response which leads to rejection.

3.2 The Immunogenicity of SCNT

To avoid rejection, the generation of autologous embryonic stem cells is considered necessary for clinical applications. The technique of SCNT has been suggested as a quick method to generate patient-specific embryonic stem cells which are genetically matched with the recipient. In brief, the nucleus from a somatic patient cell is transferred to an enucleated donor oocyte. This method was first successfully conducted in mice followed by the first human SCNT in 2013 (Tachibana et al. 2013). However, SCNT cells contain mitochondrial DNA (mtDNA) from the donor oocyte and thus carry allogeneic mitochondrial proteins. As our previous study has shown, these few mismatched minor antigens (miAg) are enough to activate the patient's immune system and cause immune rejection (Deuse et al. 2015). Thus, there is a risk that SCNT-derived cellular grafts will be rejected.

3.3 The Immunogenicity of iPS Cells

The successful reprograming of somatic cells into embryonic-like cells in 2006 (Takahashi and Yamanaka 2006), and hence the discovery of iPS cells, was a breakthrough in the stem cell world. Once generated, iPS cells can be differentiated into many cells types including cardiomyocytes, which provide an unlimited source for research and clinical applications. The biggest advantage of these cells is that they are completely genetically identical to the individual they were generated from. Thus, iPSC-derived grafts should theoretically not cause any rejection. But different studies have identified a rejection after syngeneic iPS cell transplantation, which was speculated to be due to an increase in the expression of certain stemness genes. This altered gene expression can be recognized by the immune system of the recipient, leading to rejection (Zhao et al. 2015). Other studies show no rejection after syngeneic transplantation of iPS cells. However, a patient-specific generation of such cells would be extremely costly and time-consuming. Furthermore, the quality of the derived iPS

cells depends on the patient's age and significantly affects the success of the iPS cell-mediated regeneration. Since the generation, validation, differentiation and purification of iPS cells also takes time, these patient-specific cells would not be readily available for acute diseases like myocardial infarction.

4. Strategies to Prevent Stem Cell Rejection

One proposed strategy to overcome the rejection of stem cell-derived grafts is the establishment of HLA[1]-typed iPSC banks. This would make HLA-matched iPS cells available for clinical application. For this purpose, iPS cells would preferably be generated from a large number of young, healthy donors to cover a wide spectrum of the HLA pool. Like in solid organ transplantations, an HLA typing would be conducted with each patient to find his or her HLA-matched iPS cell line. But even this method is not a solution to overcoming the immunobiological hurdle because even full HLA-matching would leave minor MHC antigens (miAgs) which are enough to cause immune rejection. A recent study on non-human primates provided conclusive proof that even full MHC matching is not enough to achieve acceptance of cellular grafts (Kawamura et al. 2016).

In solid organ transplantation, acute rejection can be avoided by using immunosuppressants. The long-term use of this treatment in cell therapy, however, is not justifiable and feasible as it can lead to severe side effects including cardiovascular complications, infections, and increased risk of cancer, among other things.

A new strategy is required to find a renewable source of cells that can be used for human therapy where allogeneic grafts are routinely accepted without any immunosuppression. We aim to create a hypo-immunogenic stem cell line that is accepted by any recipient. This kind of "off-the-shelf" cell line could be put to clinical use for cell therapy in regenerative medicine, not just for cardiovascular diseases but also for any other application where stem cell therapy is the ideal option.

5. An Example from Nature: Fetomaternal Tolerance

Fetomaternal tolerance is a natural example of such a model. During pregnancy, the fetus has mixed maternal and paternal tissue antigens and generates a temporary state of tolerance that prevents maternal immune activation. The fetus represents a foreign entity to the maternal immune system, yet this "allograft" is not rejected. Our aim is to utilize the mechanism of fetomaternal tolerance to generate universally acceptable hypo-immunogenic cells.

5.1 The Immune Features of Fetomaternal Tolerance

Trophoblast cells have been shown to have a low expression of MHC class I molecules, which are believed to be extremely important for allo-recognition (Fig. 1). Low MHC class I may contribute to the vastly elevated cytotoxic T cell activation observed in multiple studies.

1 HLA – Human leukocyte antigen.

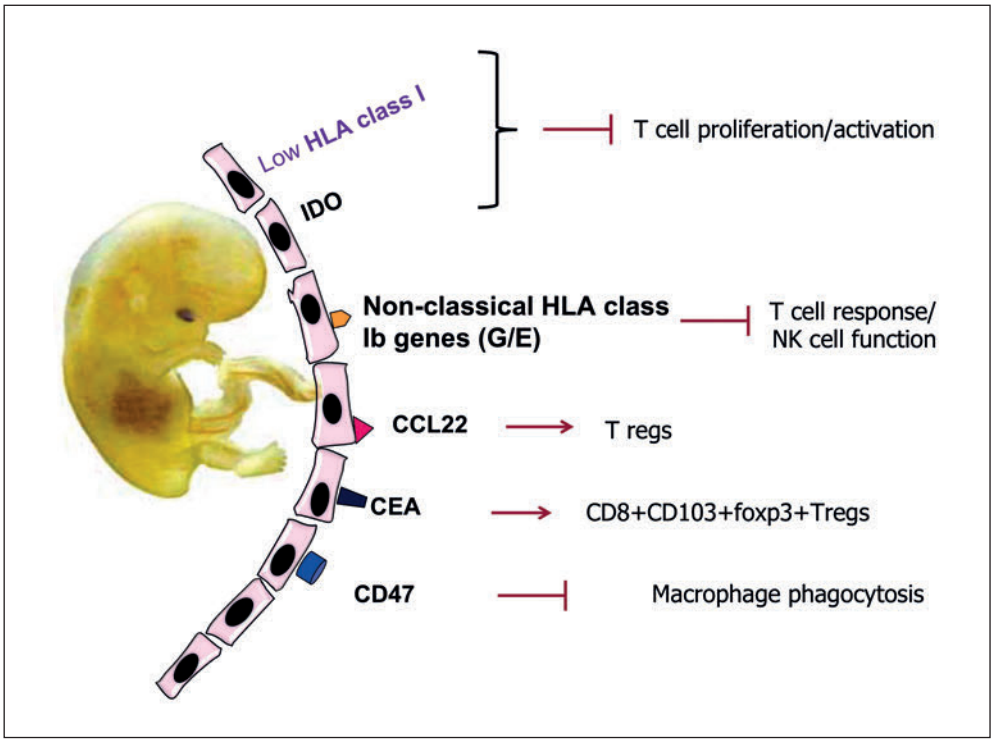

Fig. 1 Fetomaternal tolerance. Multiple mechanisms have been proposed that would contribute to fetomaternal tolerance, although their individual roles are not yet well defined.

Indoleamine-2,3-dioxygenase (IDO) is an enzyme that cleaves tryptophan, an essential amino acid for T cell proliferation, and converts it to N-formyl-L-kynurenine. It is highly expressed in trophoblasts in mice and humans, where it could possibly inhibit maternal T cell proliferation by depriving T cells of tryptophan. Studies have shown that tryptophan levels in serum decrease after the first trimester of pregnancy. The inhibition of IDO activity during murine pregnancy has led to maternal T cell-based rejection of allogeneic but not of syngeneic concepti (Santillan et al. 2015). Other studies also suggest the induction of IDO expression by regulatory T cells (Tregs) (Lee et al. 2015). IDO also indirectly influences immune activation by altering dendritic cells (DCs) by decreasing the function of antigen presenting cells (APCs) (Huang et al. 2010) or by upregulating the expression of suppressive ligands (e.g. CD95 ligands).

The non-classical HLA molecules G and E are believed to be immunomodulatory, and strong HLA G and E expression was found in trophoblast cells. These special HLA molecules have been associated with tolerance rather than rejection and may represent another mechanism of fetomaternal tolerance.

Additional molecules, like CCL22 or CEA, are being evaluated for their role in attracting regulatory T cells. The accumulation of regulatory immune cells surrounding the trophoblast suggests that they contribute to building tolerance. CD47, a molecule that interacts with innate phagocytic function, may also play a role in this context.

5.2 Engineering Hypo-Immunogenic Stem Cells

Various gene editing tools can be used to engineer immunologically modified stem cell lines. We are currently using the CRISPR/Cas9 technology, which allows us to precisely knockout specific genes and to achieve over-expression of other genes. It is not yet clear which of the described molecules are most important and should be included in the engineering plan. We are currently evaluating the immunogenicity of MHC class I knockout, IDO overexpression, and CD47 knockout.

5.3 How Do Cells Survive after Transplantation?

Our previous work (Deuse et al. 2011) has already provided proof of the efficacy of MHC class I knockdown. Studies that include other gene modifications are ongoing. In addition to hypo-immunogenicity, the utilization of a scaffold may further facilitate cell survival. Such scaffolds could be hydrogel-based and contain fibrin or collagen, and could create a protective environment for cellular grafts.

6. Conclusion

The clinical application of stem cell therapy techniques is ongoing and developing quickly. The potential of having unlimited cell sources to replace damaged tissue is huge. However, the immunogenicity of stem cells remains a major problem for the long-term survival of cellular grafts. In this overview, we introduced a new method for avoiding rejection after stem cell transplantation by generating hypo-immunogenic stem cell lines which could prevent the activation of the recipient's immune system. If successful, the next steps in a translational approach towards clinical application may include the differentiation of hypo-immunogenic stem cells into hypo-immunogenic cardiomyocytes and their expansion. Cardiomyocytes could be transplanted into the myocardium of patients receiving LVADs (left ventricular assist devices) in order to investigate their safety. If those patients receive a heart transplant later, ES cell-derived cardiomyocyte engraftment could be investigated. We believe that our approach is feasible and realistic given recent discoveries by us and other groups (Deuse et al. 2011, Swijnenburg et al. 2008a, b). Although our current research mostly relies on animals (mice), we believe that it can be quickly translated to humans. If successful, it will address a pressing clinical need and provide hope that there will be increased success in stem cell therapy.

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Vascular Regeneration and Repair in Cardiovascular High-Risk Patients

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Abstract

It has been a vision to use cellular repair to restore function after cardiovascular events such as myocardial infarction or heart failure. Vascular regeneration is brought about by the release of progenitor cells from bone marrow. Cardiovascular risk factors, like hypertension, diabetes and smoking, have been shown to reduce the number and migratory function of endothelial progenitor cells (EPCs). Mobilization of EPCs can be used as a therapeutic intervention to restore vascular integrity and function in high-risk patients.

Zusammenfassung

Es ist eine langjährige Vision, die Zellreparatur für die Wiederherstellung der Funktion nach kardiovaskulären Ereignissen, wie Myokardinfarkt oder Herzinsuffizienz, zu nutzen. Eine vaskuläre Regeneration wird möglicherweise durch die Freisetzung von Stammzellen aus dem Knochenmark ermöglicht. Kardiovaskuläre Risikofaktoren, wie Bluthochdruck, Diabetes und Rauchen, reduzieren die Anzahl und Wanderfunktion von endothelialen Stammzellen (EPCs). Die Mobilisierung der endothelialen Stammzellen kann eingesetzt werden, um die vaskuläre Intaktheit und Funktion in Hochrisikopatienten wiederherzustellen.

1. Introduction

It has been a long-standing vision to use cellular repair to regenerate terminally differentiated tissues, like myocardial and vascular structures, and to restore function after cardiovascular events such as myocardial infarction or heart failure. There is evidence that complete heart regeneration is possible in zebra fish (Poss et al. 2002), and snakes exhibit postprandial cardiac adaptation with hyperplasia and hypertrophy (Andersen et al. 2005). The first evidence of myocardial regeneration in humans came from chimerism studies, which found varying degrees of cardiomyocyte regeneration in human hearts after counter-gender transplantation, with myocyte regeneration rates of 0.04 % (Laflamme et al. 2002), 0.3 % (Bayes-Genis et al. 2002), 1.5 % (Müller et al. 2002), and 18 % (Quaini et al. 2002). Recent experimental data in dogs have indicated the possibility of trans-differentiation into endothelial cells, myocytes and smooth muscle cells from cardiac stem cell niches (Linke et al. 2005). The idea of significant myocardial regeneration has recently been challenged, indicating that the concept and therapeutic efficacy still remain inconclusive (Orlic et al. 2001, Murry et al. 2004, Balsam et al. 2004).

2. Vascular Regeneration

Vascular regeneration is thought to be brought about by the release of progenitor cells from bone marrow (Asahara et al. 1997, Rosenzweig 2003). Indeed, cardiovascular risk factors, like hypertension, diabetes and, in particular, smoking, have been shown to reduce the number and migratory function of endothelial progenitor cells (EPCs) (Perticone et al. 2001). The number of EPCs inversely correlates to cardiovascular death and the composite of cardiovascular death, cardiovascular hospitalization, myocardial infarction and urgent revascularization (Werner et al. 2005). It is also related to endothelial function measured by forearm blood flow (Hill et al. 2003). The correlation between endothelial function and EPCs has also been associated with erectile dysfunction, primarily in high-risk patients. This is mediated by endothelial dysfunction (Baumhäkel et al. 2006) and is also related to cardiovascular outcomes (Böhm et al. 2010).

The question thus arises as to whether endothelial progenitor cell numbers and function can be used as a therapeutic intervention to restore vascular integrity and function. In a "response to injury" model using endothelial damage of the carotid artery, injected green fluorescent proteins (GFPs) have been shown adjacent to the site of endothelial damage and to

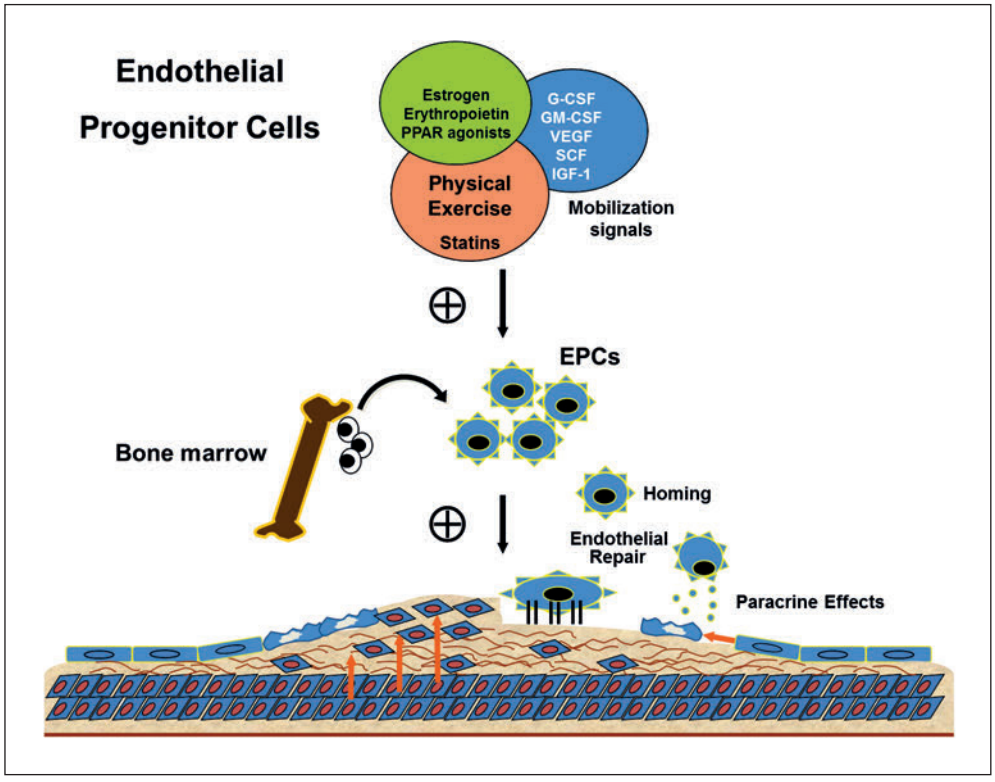

Fig. 1 Illustration of endothelial progenitor cell (EPC) mobilization mechanisms and their effects. Abbreviations: VEGF: vascular endothelial growth factor, SCF: stem cell factor, GM-CSF: granulocyte macrophage colony-stimulating factor, G-CSF: granulocyte colony-stimulating factor, IGF-1: insulin-like growth factor 1.

completely cover the lesions with cells expressing von Willebrand factor. This data provides evidence that EPCs can integrate into vessels and transdifferentiate into fully functioning endothelial cells (Werner et al. 2003). Cardioprotective drugs, like statins, were able to increase the number of EPCs and to reduce neointima formation in this model (Werner et al. 2002). Physical exercise has been shown to be linked to an increased number of EPCs and to reduce neointima formation while enhancing angiogenesis (Laufs et al. 2004). In a model of an experimental stroke, physical activity was accompanied by a reduction in the magnitude of the stroke, an increase in EPCs, and an improvement in endothelial function (Gertz et al. 2006). These EPC effects were reproduced in patients with high cardiovascular risk, according to controlled protocols of physical exercise (Gertz et al. 2006). Figure 1 summarizes EPC mobilization mechanisms and their effects.

3. Endothelial Reparation and Restoration in Myocardial Remodeling

Pressure overload is an important cause of relaxant and contractile dysfunction and can lead to heart failure with preserved ejection fraction. There is a mismatch of endothelial cells to myocytes, indicating an impaired myocardial energy delivery as part of this condition. A pressure overload model has shown that endothelial and myocardial progenitor cells are upregulated, most likely as a compensatory mechanism. This effect appeared to be mediated by endothelial nitric oxide, which also promotes angiogenesis. Resident cardiac stem cells are upregulated in response to pressure overload and are able to reduce myocardial fibrosis and, therefore, provide a target for medical treatment. Some traditional heart failure drugs, like ACE inhibitors, also mediate EPC activation. This could explain their beneficial effects in cardiovascular high-risk patients and heart failure.

4. Summary and Perspectives

The topic of cardiomyocyte regeneration in the heart remains debatable. In particular, the number of regenerated myocytes might not be able to sufficiently compensate for the loss of myocytes after pressure overload or myocardial infarction. Endothelial progenitor cells have been shown to be downregulated by cardiovascular risk factors. Their application reduces cardiovascular lesions in experiments. Drugs, like statins and ACE inhibitors, as well as physical exercise can enhance their function and numbers, thereby reducing vascular damage and improving endothelial function. Finally, EPCs in cardiac stem cells have been shown in experiments to improve myocardial remodeling processes by enhancing angiogenesis, endothelial function and by inhibiting myocardial apoptosis. These mechanisms and further research will hopefully identify new mechanisms that prevent cardiovascular events and treat vascular disease and heart failure.

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Human Rights and Refugees

Nova Acta Leopoldina N. F. Nr. *415*
Herausgegeben von Hans-Peter Zenner (Tübingen) und Alenka Šelih (Ljubljana, Slowenia)
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Das *Human Rights Committee (HRC)* der Nationalen Akademie der Wissenschaften Leopoldina besteht aus Akademiemitgliedern aus Deutschland, Österreich und der Schweiz. Es unterstützt Wissenschaftlerinnen und Wissenschaftler, die von Unterdrückung und Einschränkungen ihrer Tätigkeit betroffen sind, durch Briefe, Stellungnahmen und die Organisation von Veranstaltungen. Die Tagung 2016, die gemeinsam mit der Slowenischen Akademie der Wissenschaften organisiert wurde, beschäftigte sich mit der Migration und Integration von Flüchtlingen und Migranten, die aus dem Nahen Osten, Afrika und anderen Teilen der Welt nach Europa kommen. Besonders wurden Fragen der Menschenrechte, Möglichkeiten eines humanen Grenzmanagements und die Rolle von Deutschland bzw. Slowenien als Gastländer analysiert.

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Myocardial Regeneration: News from Bench and Bedside

Gerd Hasenfuss (Göttingen)

Abstract

A large number of experimental and clinical studies have been performed with the aim of developing new strategies for treating patients after myocardial infarction and heart failure. Bone marrow cells were investigated in an initial phase, followed by cardiac-derived stem cells examined as part of a second phase. It has been suggested that these cells may influence cardiac remodeling processes, due to paracrine actions, without transdifferentiating into myocytes. While the results of clinical trials have been controversial, large outcome trials are pending. The third phase of stem cell therapy is based on human pluripotent stem cells, either from embryonic stem cells or from induced pluripotent stem cells (iPSCs). Even though this third phase is still in its early stages, preliminary findings are promising, in particular when these cells are used in tissue engineering.

Zusammenfassung

Eine große Anzahl von experimentellen und klinischen Studien ist mit dem Ziel durchgeführt worden, neue Strategien für die Behandlung von Patienten nach Mykardinfarkt und Herzinsuffizienz zu entwickeln. Knochenmarkzellen wurden in der Initiationsphase untersucht, gefolgt von aus dem Herz hervorgehenden Stammzellen in einer zweiten Phase. Es ist vorgeschlagen worden, dass diese Zellen den kardialen Remodellierungsprozess, aufgrund parakriner Aktivitäten, ohne Transdifferenzierung in Myozyten, beeinflussen. Während die Ergebnisse der bisherigen klinischen Studien umstritten sind, stehen umfassendere Ansätze mit Sterblichkeitsendpunkten noch aus. Die dritte Phase der Stammzelltherapie basiert auf humanen pluripotenten Stammzellen, entweder aus embryonalen Stammzellen oder aus induzierten pluripotenten Stammzellen (iPSCs). Obwohl sich diese dritte Phase noch in den Kinderschuhen befindet, sind vorläufige Resultate verheißungsvoll, insbesondere wenn die Zellen im Tissue-Engineering-Ansatz genutzt werden.

1. Introduction

Myocardial regeneration strategies are being developed to treat chronic heart failure and/or to prevent remodeling and heart failure from developing after myocardial infarctions. Goals can be achieved by generating new cardiomyocytes or by chemically influencing processes such as angiogenesis, inflammation or apoptosis. The latter may also be cell-mediated (cell-based pharmacotherapy). New cardiac myocytes can be generated in the following ways: (*1*) by inducing the proliferation of existing myocytes, (*2*) by reprogramming fibroblasts into contracting myocytes, (*3*) by activating stem cells to differentiate into cardiomyocytes thereby cells may be recruital from within the heart or from extracordiac tissues.

2. Developments – First Phase

Since 2001 major research efforts have addressed external stem cell applications. Orlic et al. (2001) were the first to show that bone marrow cells from mice, which are transplanted into rat hearts after myocardial infarction, differentiate into myocytes and vascular cells resulting in improved function. A number of subsequent studies revealed similar findings. However, in 2004 various publications convincingly demonstrated that bone marrow cells do not transdifferentiate into cardiac myocytes. Nygren et al. (2004) identified cell fusion but no transdifferentiation, Murry et al. (2004) demonstrated that hematopoietic stem cells do not transdifferentiate into cardiac myocytes, and Balsam et al. (2004) had similar findings yet observed a slight improvement in cardiac function.

Driven by Orlic (2001) and others, a number of clinical studies were conducted that included the intracoronary injection of bone marrow-derived cells or the intramyocardial injection of cells. Most of these studies did not have randomized, blind designs, and their results were heterogeneous. The BOOST trial, generally considered to be a carefully performed study, observed an initial improvement in left ventricular function in patients receiving transplanted bone marrow cells compared to the control group (Wollert et al. 2004). However, after 18 months and by the 5-year, long-term follow-up, differences between the control and transplant group had disappeared (Meyer et al. 2006).

This first phase of stem cell therapy may be summarized as follows: bone marrow-derived cells generally do not transdifferentiate into intact cardiomyocytes. Some beneficial effects on myocardial function after bone marrow cell transplantation may yet result from the paracrine activity of these cells; however, a long-term effect on myocardial function is questionable.

3. Developments – Second Phase

In the second phase of stem cell therapy, approaches were developed that used stem cells derived from the adult heart. Bearzi et al. (2007) showed that c-kit$^+$ cells can be derived from human myocardial biopsies. The authors further demonstrated that injection of these cells into mouse hearts after myocardial infarction regenerate new myocytes and capillaries. Another approach used so-called cardiospheres, heterogeneous populations of cells obtained from atrial and ventricular biopsies after a number of culture steps (Messina et al. 2004). Both cell strategies and a number of other cardiac-derived cell types have been studied in clinical trials. In subsequent years, a number of meta-analyses were published, but even these meta-analyses were inconsistent. Gyöngyösi et al. (2015) concluded in their meta-analysis of individual patient data from randomized trials of patients with recent acute myocardial infarction that intra-coronary cell therapy provided no benefit in terms of clinical events or changes in left ventricular function, and these analyses included data from 12 randomized trials. However, in the same year Fisher et al. (2015) came to the conclusion from their meta-analysis that "this study shows evidence that autologous cell therapy may be beneficial for patients having heart failure". Reasons for the discrepant findings are not clear. They may be related in part to different patient populations: acute myocardial infarction versus chronic heart failure.

In a critical analysis, Francis et al. (2013) suggested that discrepancies and contradictions of clinical trials on stem cell therapy may result from differences in trial design, inconsistencies in data presentation, and superficiality of data presentation. In fact, Nowbar et al. (2014) suggested that there is a correlation between the trial's effect size and the number of discrepancies per trial. For example, Bolli et al. (2011) published the Scipio trial: "Cardiac stem cells in patients with ischemic cardiomyopathy (SCIPIO): initial results of a randomized phase 1 trial". In 2014, editors of the *Lancet* published an expression of concern: On March 25, 2014, Gretchen Brodnicki, Harvard Medical School's Dean of Faculty and Research Integrity, wrote to *The Lancet* to inform us that "Harvard Medical School (HMS) and Brigham and Women's Hospital (BWH) are reviewing concerns about the integrity of certain data generated in a laboratory at BWH and included in the SCIPIO paper".

Considering these tremendous uncertainties and inconsistencies, a significant number of researchers doubt that bone marrow cell treatment or cardiac-derived cell-based therapies will have a future in clinical medicine. However, a number of trials are ongoing, and we should await their results before a final conclusion is drawn.

4. Developments – Third Phase

The third phase of stem cell therapy is based on human pluripotent stem cells, such as human embryonic stem cells or induced pluripotent stem cells (iPSCs). Chong et al. (2014) showed that neonatal-type cardiomyocytes, generated from existing human embryonic stem cells, could be safely transplanted into non-human primates after myocardial infarction. Gerbin and Murry (2015) showed that human embryonic stem cells generate new cardiomyocytes after intramyocardial injection which couple to existing myocytes. Furthermore, Menasché et al. (2015) showed that human embryonic stem cell-derived cardiac progenitors were safely transplanted as a fibrin patch into a patient's pericardial pocket, with subsequent improvement of function and patient performance. Other strategies make use of iPSCs, which can be derived by reprogramming any differentiated human cell into a pluripotent cell with subsequent differentiation into cardiomyocytes. Takahashi et al. (2007) demonstrated that pluripotent stem cells from adult human fibroblasts could be reprogrammed using defined factors. Yamanaka received the Nobel Prize in 2014 for this innovation. Since 2007, reprogramming techniques have been modified and improved, and a number of tissue engineering approaches have been developed that use these autologous/allogenic cells to replace scar tissue or to support failing hearts.

We were recently able to show that engineered heart muscle (EHM) from human embryonic cells or iPSCs exhibits the properties of natural myocardium and is able to repair damaged hearts (Tiburcy et al. 2017). We speculate that tissue engineering based on human embryonic stem cells or, based on induced pluripotent stem cells from humans, may have a great potential for regenerative strategies in heart failure patients.

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Symmetrie und Asymmetrie in Wissenschaft und Kunst

Vorträge anlässlich der Jahresversammlung
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Symmetrien und Asymmetrien gibt es in allen Bereichen der Wirklichkeit. Das Bestreben, ihr Wechselspiel zu erfassen, verbindet die Wissenschaften mit den Künsten. Symmetrie und Asymmetrie sind Strukturprinzipien, die sich in Mathematik und Philosophie abstrakt definieren lassen, aber auch in den empirischen Wissenschaften eine Vielzahl von Phänomenen in Natur und Kultur erklären können. Doch auch die Kunst – von der Musik bis zur Architektur – beschäftigt sich in jeweils eigener Weise mit Symmetrie und Symmetriebrüchen. Der Band liefert Beispiele, die von der Physik, insbesondere der Teilchenphysik, über die Astronomie, die Chemie (z. B. Chiralität) und die Biologie (z. B. Fortbewegung) bis in das Gebiet der Medizin (z. B. Krebsentstehung und Wundheilung) reichen. Außerdem werden Symmetriebrüche in der Moral, die Rolle von Symmetrie und Asymmetrie in der bildenden Kunst und die Symmetrie als strukturelles Prinzip des Denkens in der Moderne untersucht.

Wissenschaftliche Verlagsgesellschaft Stuttgart

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Regeneration of the Heart

Bernd K. Fleischmann ML (Bonn)

Abstract

Cardiovascular diseases are among the most frequent causes of death. In particular major heart infarctions with severe heart failure have a poor prognosis, and heart transplantation is currently the only causal treatment available. We are therefore testing exogenous and endogenous repair approaches and are taking advantage of the expression of optogenetic molecules in cardiac cells to modulate the electrical activity of the heart *in vivo* and *ex vivo* by applying blue light pulses.

Zusammenfassung

Kardiovaskuläre Krankheiten zählen zu den häufigsten Todesursachen weltweit. Insbesondere große Herzinfarkte mit Herzinsuffizienz haben eine schlechte Prognose, und die einzige kausale Therapie ist die Herztransplantation. Wir interessieren uns für die Herzregeneration und testen exogene- und endogene Reparaturansätze. Darüber hinaus exprimieren wir optogenetische Moleküle im Herzen und zeigen, dass dadurch die elektrische Aktivität des Herzens mit blauem Licht moduliert werden kann.

1. Regeneration of the Heart

In my talk I have tried to summarize key aspects of heart regeneration, in particular potential strategies to enhance repair, as well as approaches to address the acute and chronic complications of heart attacks.

Since cardiovascular diseases are the most frequent cause of death, there is great interest in reducing its morbidity and mortality rates. One of the most frequent and serious events is a myocardial infarction. Invasive procedures and drug treatment have greatly improved survival rates. However, patients with major myocardial infarcts often suffer from severe heart failure and have a relatively low life expectancy. The only causal treatment currently available is heart transplantation, but the number of patients in need greatly outnumbers available organs. Assist devices are a short-to-medium-term option. Nevertheless, alternative approaches are needed to enhance cardiac pump function over the long term. Since experimental evidence demonstrates that there is very little, if any, regenerative capacity in the adult mammalian heart muscle (Bergmann et al. 2009, 2015), exogenous and endogenous repair approaches are interesting strategies for improving long-term post-infarct heart function.

2. Exogenous Repair

The idea of this approach is to bring contractile cells into the lesioned area and have them engraft and contribute to contraction. Many studies have been conducted that use mesenchymal stem cells derived from bone marrow. The initial claim was that these cells transdiffer-

entiate into functional cardiomyocytes and endothelial cells. This prompted the initiation of clinical trials using autologous bone marrow cells from heart attack patients which yielded contrasting results. Our group collaborated with Sten Eirik Jacobsen's group to perform a detailed analysis of the fate of transplanted and mobilized bone marrow (BM) cells in infarcted mice. We found no evidence for the transdifferentiation of these cells (Nygren et al. 2004, Kolossov et al. 2006), however we were able to identify rare heterologous cell fusion events (Nygren et al. 2004, 2008) which could be one of the reasons behind the controversial findings and interpretation of the data. Overall, there is consensus in the field that BM cells do not transdifferentiate into functional cardiomyocytes and that potential beneficial effects observed in experimental studies and clinical trials are due to the paracrine effects of these cells. We are pursuing the idea that pluripotent stem cells are a valuable source for generating large amounts of early cardiomyocytes, which can be transplanted into the infarction. We have explored this approach in a proof of concept study and investigated, in particular, whether it is possible to avoid teratoma formation as a result of contaminating undifferentiated stem cells. In fact, even co-transplantation of relatively low numbers of contaminating ES cells resulted in tumor formation (Kolossov et al. 2006). We therefore used genetically modified, stably transfected mouse ES cells where the αMHC promoter drives expression of the puromycin resistance gene. Application of puromycin to the growing and differentiating ES cells led to a strong enrichment of cardiomyocytes (> 99 %) within a few days and the eradication of undifferentiated ES cells. Intramyocardial injection of approx. 100,000 to 200,000 ES cardiomyocytes into freshly infarcted mouse hearts yielded an engraftment for many weeks and even improved left ventricular function (Kolossov et al. 2006). We are currently trying novel approaches in order to mechanistically better understand and reduce the steady loss of grafted cardiomyocytes over time, since fewer than 5 % of the injected cells can be re-found after 2 weeks.

3. Endogenous Repair

Our recent efforts are directed at investigating potential endogenous repair mechanisms in the mammalian heart and to manipulate these to enhance cardiac regeneration. A long-standing question is whether the adult heart harbors cardiac stem cells and whether these are activated to regenerate cardiomyocytes after a lesion. Most publications proposed that there are c-kit$^+$ stem cells within the adult mammalian heart that display cardiomyogenic potential. We therefore collaborated on this topic with M. Kotlikoff's group, taking advantage of a c-kit-EGFP[1] transgenic mouse model. We could identify a few EGFP$^+$ cardiomyocytes during late embryonic and early postnatal stages. When isolating single ckit$^+$ cells from the postnatal heart and investigating their fate, we could observe that very few of these expanded and gave rise to cardiomyocytes, endothelial cells (ECs) and smooth muscle cells. These findings are in line with the concept of multipotent stem cells. However, cells with this potential could not be found in the adult heart. In fact few ckit$^+$ cells became visible in the border zone and within the infarct area and the cells appeared to preferentially label ECs, potentially indicating their contribution in re-vascularization (Tallini et al. 2009). These findings prompted us to probe whether multipotent ckit$^+$ cells could potentially contribute to heart repair in neonatal hearts, and we established a cryoinjury model for this purpose (Jesty et al. 2012). Within hours after

1 EGFP – Enhanced Green Fluorescent Protein.

induction of the lesion we found a strong upregulation of early genes of embryonic cardiomyogenesis in the hearts, prominent ckit-EGFP expression in the cardiomyocytes, and ECs and other cells in the border zone and the infarct area. Moreover, we observed prominent neo-myogenesis and scar size was found to be relatively small (Jesty et al. 2012) at 3 months after the lesion. When investigating the cellular source of the neomyogenesis using a fate mapping approach, we found that the large majority originated from resident and proliferating cardiomyocytes, whereas a much smaller proportion appeared to be derived from c-kit-EGFP$^+$ cells (Jesty et al. 2012). Thus, multipotent ckit$^+$ cells appear to be involved in the repair of the neonatal heart, although these cells are not present in the adult heart, and the contribution of ckit$^+$ cells appears to be largely restricted to revascularization (Hesse et al. 2014).

Because we found that endogenous stem cells do not play an important role in the injured adult murine heart we then investigated the cell cycle activity in cardiomyocytes. The numbers of cycling cardiomyocytes in uninjured hearts and in hearts after injury vary greatly in the literature. This is most likely due to the fact that cardiomyocytes display atypical cell cycle activity similar to hepatocytes, namely acytokinetic mitosis and endoreduplication. Both of these processes result in increased cellular DNA content and therefore positive labeling by classic cell cycle markers such as Ki-67 or Bromdesoxyuridin (BrdU), however, they do not result in cytokinesis and an increased number of cardiomyocytes. We therefore generated a transgenic system in which the M-phase of the cell cycle could be monitored by the different subcellular localization of the *live* reporter EGFP (Hesse et al. 2012). A transgene was established for this purpose, where anillin, a component of the contractile ring, was fused to EGFP and expressed under the control of a ubiquitous promoter. In non-cell cycle active cells, the fusion protein is ubiquitinated by the APCCdh1[2] complex and degraded in the proteasome. This ensures specificity of the signal. After establishing stable ES cell lines and testing the function of the construct, we generated transgenic mice using complementation assays with diploid and tetraploid embryos. We were unable to detect EGFP-anillin$^+$ cardiomyocytes in the adult transgenic mouse heart, but after cardiac lesions we found a few cardiomyocytes displaying EGFP$^+$ nuclei, but no signs of M-phase, as no contractile rings or midbodies were visible. Therefore, we then quantified DNA content and found that these EGFP-anillin$^+$ cardiomyocytes were endoreduplicating (Hesse et al. 2012), similar to what is suggested with human cardiomyocytes after cardiac lesion (Herget et al. 1997, Meckert et al. 2005). Thus, using a novel transgenic model, our data demonstrates that very little, if any, regeneration of cardiomyocytes can be observed in the adult mouse heart after cardiac lesion. Nevertheless, the directed induction of cardiomyocyte proliferation and cell division would be an ideal approach for adult heart repair. Like other groups, this prompted us to test signaling pathways and low molecular weight substance libraries to identify factors that have the potential to induce authentic cell division in resident adult cardiomyocytes. Thus, we established a novel screening assay for cardiomyocyte cell cycle activity and cytokinesis by crossing the EGFP-anillin mouse line with another established model in the laboratory, in which the αMHC-promoter drives the expression of the H2B-mCherry transgene (Raulf et al. 2015). In this mouse model, all cardiomyocytes carry red fluorescent nuclei and can be easily recognized under fluorescent light. The proliferation assay is based on the isolation of neonatal double transgenic heart cells. Different factors and substances can be added to the plated cells and, a few days later, changes in the rate of proliferating and dividing cardiomyocytes can be easily detected and quantified using a fluorescence microscope (Raulf et al. 2015).

2 APCCdh1 – Anaphase Promoting Complex.

4. Modulation of the Electrical Activity of the Mouse Heart using Optogenetics

The heart is characterized by its electrical automatism which is due to the presence of specialized, spontaneously active cells. The heart is also an electrical syncytium because it expresses connexins. Arrhythmias are relatively frequent syndromes; in the case of severe heart disease they are one of the most frequent and potentially lethal complications. Even though pacemakers and automatic defibrillators are well established and routinely used in patients, alternative ways to modulate the heart's electrical activity is of interest to basic research and to experimental therapeutic applications. We have therefore assessed the utility of optogenetic molecules, in particular channelrhodopsin-2 (ChR2). This molecule is a non-selective cation channel that changes its confirmation upon exposure to blue light. This enables preferentially Na^+ ions to influx into the cell based on the electrochemical gradient and leads to depolarization of the membrane potential. We initially tested the utility of ChR2 in stably transfected ES cells under the control of an ubiquitous promoter. During the differentiation of the ES cells, we witnessed unsynchronized spontaneous beating of ESC-derived cardiomyocytes. The application of short blue light pulses led to the synchronous beating and contraction of these transgenic ESC-derived cardiomyocytes (Bruegmann et al. 2010). Interestingly, long-term blue light exposure led to extended depolarization of the cardiomyocytes. We generated transgenic mice from these ES cells and found that the transgenic ChR2 expressing mouse heart could be paced *ex vivo* and *in vivo* by blue light pulses (Bruegmann et al. 2010). This technology enables the cellular mechanisms underlying arrhythmia generation in the atrium and the ventricle to be investigated. We next tested whether ChR2 could also be expressed in adult hearts using viral technologies and applied a single injection of an AAV-9 vector into the jugular vein where a ubiquitous promoter drives the expression of ChR2. After several weeks, we found that more than 50 % of cardiomyocytes expressed ChR2 and that single cells as well as most of the hearts could be paced upon exposure to blue light (Vogt et al. 2015). Heart defibrillation could be one possible application of this technology. To this end, an *ex vivo* model based on short QT-duration was established, where long lasting ventricular tachycardia (VT) could be induced. We found that applying blue light to the left ventricles of hearts expressing either transgenic or AAV9-transduced ChR2 could interrupt VTs. This effect was found to depend on light intensity, duration of the pulse and the surface area of the light exposure (Bruegmann et al. 2016). Taken together, this data demonstrates that the use of optogenetic molecules makes it possible to pace mammalian hearts and to alter its pathological electrical activity.

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Crossing Boundaries in Science: Modelling Nature and Society – Can We Control the World?

Nova Acta Leopoldina N. F. Nr. *419*
Herausgegeben von Johannes Fritsch (Berlin), Yvonne Borchert (Berlin)
und Jörg Hacker (Halle/Saale)
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Der im Band referierte Workshop „Modelling Nature and Society – Can We Control the World?“ bildet den Auftakt zu einer neuen Veranstaltungsreihe „Crossing Boundaries in Science“ der Leopoldina, zu deren zentralen Aufgaben als Nationaler Akademie der Wissenschaften es gehört, Politik und Öffentlichkeit zu gesellschaftlich bedeutsamen Fragen zu beraten und Bezüge zwischen Wissenschaft, Forschung, Politik und Gesellschaft zu vermitteln. Neue Forschungsgebiete sollen daher frühzeitig identifiziert, ihre zukünftige Entwicklung und gesellschaftliche Nutzung diskutiert werden. Ziel ist es, in besonderem Maße zur interdisziplinären Zusammenarbeit anzuregen und damit die Diskussionen um Wissenstransfer und die Anwendungen neuer Technologien zu befördern.

Im Mittelpunkt des Bandes steht die Modellbildung von komplexen Systemen in verschiedenen Bereichen der Wissenschaft, insbesondere in biologischen und sozialen Netzwerken. Die analysierten Themen reichen von Gen-Netzwerken und Fragen des Immunsystems bis hin zu technischen Systemen, z. B. Verkehrssystemen, und komplexen wirtschaftlichen Fragestellungen, bis hin zur globalen Ebene.

Wissenschaftliche Verlagsgesellschaft Stuttgart

Nova Acta Leopoldina NF Nr. *416*, 51–53 (2017)

Modeling Human Genetics in Induced Pluripotent Stem Cells (iPCs) for Cardiac Disease

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Abstract

Numerous studies have led to exciting new insights into the progress of modeling in the cardiovascular field. This short contribution will focus on the impact of the genetic background on the use of human induced pluripotent stem cells (hiPSCs) in modeling cardiac disease and on the differentiation of cardiomyocyte subtype lineages.

Zusammenfassung

Zahlreiche Studien haben zu aufsehenerregenden neuen Erkenntnissen auf dem Gebiet der Modellierung kardiovaskulärer Erkrankungen geführt. Der kurze Beitrag konzentriert sich auf den Einfluss des genetischen Hintergrunds der Verwendung humaner induzierter pluripotenter Stammzellen (hiPSCS) in der Modellierung kardiovaskulärer Erkrankungen und auf die Differenzierung von Kardiomyozyten-Subtypen-Linien.

1. Introduction

Recent years have witnessed great progress in iPSC-based disease modeling in the cardiovascular field, and numerous studies have led to exciting new insights into the molecular mechanisms underlying various cardiac diseases. Moreover, the use of induced pluripotent stem cells (iPCs) in drug discovery and toxicity testing is at our doorstep (Bellin and Mummery 2016, Goedel et al. 2017). However, in order to harness the entire potential of this new technology, it is essential to develop appropriate and robust assays. The following contribution will focus on two major challenges associated with the use of human induced pluripotent stem cells (hiPSCs) in modeling cardiac disease.

2. Genetic Background

One challenge is the genetic background variability of different cell lines, which may confound the analysis of the disease phenotype when comparing iPSCs from patients and independent, healthy controls. It may also influence the drug response when these cells are used for pharmacological screening or drug safety assays. This is of even greater importance when the genetic variant studied does not lead to a large change of the phenotype. To account for this fact, the number of patient and control lines in the study could be increased or well-matched controls (e.g. gender-, age-, and ethnicity-matched) could be used (Musunuru et al. 2014). Nevertheless, the phenotypic readouts would still be confounded by subtle differences in the genetic background of the different cell lines. A promising solution to this problem is

the use of isogenic control cell lines, generated using precise genome editing tools that either introduce or remove the variant under investigation in one cell line (Bellin et al. 2013). This allows the effect of this variant to be studied in an identically genetic setting. However, this approach is only feasible if the causative genetic variant is known, which might not always be the case especially when studying polygenetic or complex diseases.

3. Modeling

While the genetic background variability discussed above is more of a general issue in hiPSC-based disease modeling, there are also challenges that are more specific to the cardiovascular field. During cardiac differentiation of pluripotent stem cells, all of the different cardiomyocyte subtype lineages (atrial-, nodal-, and ventricular-like cells) emerge (Moretti et al. 2010a). These cardiomyocyte subtypes differ considerably in their electrophysiological properties, and it is almost impossible to predict which subtype will be functionally affected by a certain mutation or drug and to what extent (Moretti et al. 2010b). Thus, all subtypes must be analyzed separately which can be tricky as these cells appear to be morphologically similar and their gene expression profile is not unique. Currently single-cell patch clamp technique is the gold standard for electrophysiological studies of hiPSC-derived cardiomyocytes. Yet, this technology is time consuming, labor intensive and challenging for automatization. The availability of voltage-sensitive dyes that enable optical action potentials to be measured has created exciting opportunities for measuring action potentials in large numbers of cells within a reasonable amount of time. Furthermore, it has recently been shown by our group and others that this can even be done in a subtype-specific manner by using genetically encoded voltage sensors (Chen et al. 2017).

4. Conclusion

As demonstrated in the examples above, scientific progress in recent years suggests that most of the challenges associated with the use of hiPSCs in drug discovery and toxicity screening can be overcome. Thus, regulatory authorities world-wide are already discussing the idea of making such screening a mandatory tool in the drug development process. The second decade of human iPSC research is on the horizon and will face some new challenges as the field moves towards studying complex diseases and genetic variants associated with low phenotypic effects.

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Arthropod-borne Infectious Diseases and Arthropods as Disease Agents in Human and Animal Health

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Nova Acta Leopoldina N. F. Nr. *411*
Herausgegeben von Thomas C. Mettenleiter (Greifswald/Insel Riems), Stefanie Becker (Hannover), Theodor Hiepe (Berlin), Richard Lucius (Berlin) und Bianca M. Bussmann (Greifswald/Insel Riems)
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Vor allem blutsaugende Arthropoden besitzen als Überträger – Vektoren – von Infektionskrankheiten des Menschen und der Tiere große medizinische und wirtschaftliche Bedeutung. Sie übertragen vor allem in tropischen und subtropischen Ländern bedeutsame Erkrankungen, wie Malaria, Chagas-Krankheit und Viehseuchen. Als Probleme des ärmeren Südens der Welt fanden sie in der Forschung der reichen Industrieländer lange nicht die erforderliche Aufmerksamkeit. Der wachsende Handel und zunehmende Reiseaktivitäten in einer sich globalisierenden Welt, aber auch der Klimawandel führen zu einer Ausbreitung der Überträger und Erreger solcher Infektionskrankheiten in bisher nicht betroffene Gebiete. Die Beiträge behandeln eine große Vielfalt von Arthropoden und die von ihnen übertragenen Krankheitserreger. Die Aufklärung der Übertragungswege und der Wechselwirkungen zwischen den (als Wirte oder Vektoren) beteiligten Organismen sowie den Erregern wird mit den Mitteln von Genomik, Transkriptomik, Proteomik und Metabolomik vorangetrieben. Obwohl der Wissensstand gerade in den letzten Jahren enorm angewachsen ist, sind noch viele wichtige Fragen offen. Sie sollen auch mit diesem Band in interdisziplinärer Zusammenarbeit von Experten der verschiedenen Fachgebiete benannt bzw. geklärt werden, um effektivere Bekämpfungsstrategien für durch Arthropoden übertragene Krankheiten entwickeln zu können und in einer sich in vielfachen Wandlungsprozessen befindlichen Welt kostengünstig verfügbar zu machen.

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Stem Cells in Ophthalmology with an Emphasis on Aniridia / PAX6 Syndrome with Limbal Stem Cell Insufficiency

Barbara Käsmann-Kellner and Berthold Seitz ML (Homburg/Saar)

Abstract

Stem cells are localized throughout the ocular tissue and provide future treatment options for many currently difficult-to-treat diseases. The eye represents a good target for emerging stem cell treatments for several reasons. The contribution delivers a general survey of diseases in focus and therapeutic options.

Zusammenfassung

Stammzellen sind auch im Augengewebe vorhanden und liefern Behandlungsoptionen für viele gegenwärtig noch schwierig zu kurierende Erkrankungen. Das Auge erweist sich aus verschiedenen Gründen als gutes Ziel für Stammzellbehandlungen. Der Beitrag gibt einen Überblick zu den im Fokus stehenden Krankheiten und den therapeutischen Möglichkeiten.

1. Stem Cells in Ophthalmology

Stem cells are functionally defined as cells that can self-renew and provide ongoing populations of identical daughter cells with the same unrestricted proliferation potential. These cells are multipotent and able to give rise to all cell lineages in a particular tissue.

1.1 Localization

Stem cells are localized throughout the ocular tissues and provide future treatment options for many currently difficult-to-treat diseases. The exact locations of the stem cells in the different compartments of the orbit are listed in the paragraph describing associated disorders. Stem cells have been identified in the following tissues:

– cornea,
– conjunctiva,
– orbit,
– eyelid,
– trabecular meshwork,
– retina,
– optic nerve (head).

1.2 Associated Disorders

The **eyelids** are made up of different tissues, which provide stability of the lids and the function of lid closure, as well as various types of glands that support tear film integrity and constitute part of the smooth surface of the eye. Stem cells have been localized in the interfollicular epidermis, sebaceous glands and hair follicles. The preaponeurotic fat pads of the upper and lower lid also contain stem cells. Defects in lid stem cells lead to problems with wound healing in the lid and to cicatrizing disease which can cause severe entropium and subsequent lesions in the cornea.

When there is a depletion of **conjunctival** stem cells, the sequelae are regarded as being a cicatrizing disease. Human bulbar and forniceal conjunctival cells have a proliferative capacity. Both the conjunctival non-goblet cells and the goblet cells have a common progenitor. Therefore, in cases of stem cell insufficiency, both cell types are damaged and result in severe scar formation of the conjunctiva, often irrespective of the physiological anatomy, and form symblephara from the bulbar conjunctiva to the tarsal conjunctiva and even to the keratinized skin around the eye. This eventually leads to a keratinization of the conjunctiva and severe ocular and lid motility disorders.

Loss of stem cell function in the **cornea** leads to a loss of transparency in the clear "window" to the eye. The stem cells are situated in the stem cell niche which is found in the limbal, i.e. marginal, part of the cornea within the so-called palisades of Vogt (PV). Congenital aniridia, which is most often caused by a mutation of the PAX6 gene, is a disease that exhibits severe limbal stem cell insufficiency. This leads to corneal opacification and neovascularization of the cornea starting in childhood, often progressing to corneal blindness. Another condition associated with limbal stem cell insufficiency are alkali or acid burns of the cornea inflicted by injuries at work.

The **trabecular meshwork** is an important tissue in glaucoma, which is defined as an elevation of the intraocular pressure leading to glaucomatous atrophy of the optic nerve, visual field defects and loss of vision.

The trabecular meshwork (see Fig. 1) regulates the drainage of the intraocular fluid which is necessary for the nourishment of the ocular structures and for the stability of the globe. It is the most important structure for regulating intraocular pressure and consists of endothelial cells and an extracellular matrix. Stem cells are present in endothelial cells and in juxtacanalicular cells of the trabecular meshwork. Inner retina and optic nerve tissues also play an important role in glaucoma (see below).

The **retina** can be divided into the inner retina and the outer retina, depending on the complex and fine network of receptors, glial cells and neurons contained in the retina.

The **outer retina** mainly contains the retinal pigment epithelium and the photoreceptors (cones that provide color and detailed vision and rods that provide night vision). A small population of stem cells reside at the edge of the retina in the so-called ciliary marginal zone. These stem cells can be induced to differentiate into every retinal cell type, including photoreceptors. Diseases associated with the loss of stem cell function in the outer retina are mostly hereditary dystrophies of the retina, such as retinitis pigmentosa (initially affecting the rods and leading to a concentric tunnel vision field defect) and macular dystrophies (initially affecting the cones, leading to a large central scotoma with loss of vision).

The **inner retina** is formed by the axons generating out of the photoreceptors and forming the optic nerve. Up to now, the exact localization of stem cells is unclear. One assumes, however, that a dysfunction of stem cells of the inner retina leads to a disturbance of the rapid

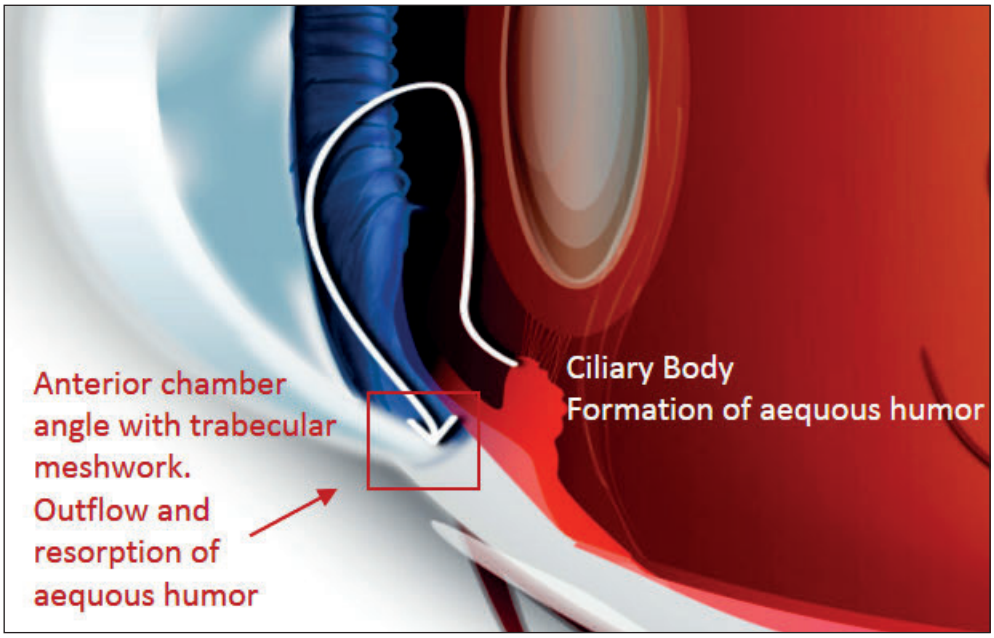

Fig. 1 Production and outflow of intraocular fluids (aqueous humor)

anterograde and retrograde axonal transport at the lamina cribrosa which is the crucial exit point of all ganglion cell axons forming the optic nerve and leading into the orbit. In stem cell dysfunction, the retinal ganglion cells are deprived of neurotrophic factors or other supportive substances. This leads to the neural damage seen in glaucoma and in other neuropathies of the optic nerve, for example hereditary optic atrophy. If the gene *optineurin* is dysfunctional, the retinal ganglion cells show a higher sensitivity to damage; this has been shown to be associated with a hereditary form of glaucoma.

In the **orbit** that surrounds and protects the eye, the source of multipotent stem cells is the adipose tissue of the nasal fat pad of the orbit. The cells can differentiate into osteogenic, adipogenic, myogenic, neurogenic and chondrogenic lineages, thus mirroring the multitude of tissues in the orbit, which contains fat (serving as an anti-shock pad for the moving eye), the eye muscles, the optic nerve, oculomotoric, sympathetic and parasympathic nerves, and cellular connective tissues. The disease associated with stem cell dysfunction in the orbit is Grave's disease, also known as endocrine thyroid orbitopathy.

1.3 Therapeutic Options

1.3.1 General Aspects of Ocular Stem Cell Treatment

The eye represents a good target for emerging stem cell treatments for several reasons:

– It is relatively self-contained and has barriers that keep cells from migrating to other parts of the body.

– Compared to other organs and tissues, it is easy to assess the effectiveness of eye treatments.
– Visual functions can be measured to evaluate different aspects of vision, such as color vision, foveal, i.e. central, visual acuity, the visual field, contrast sensitivity, motion perception and vision in dark surroundings.
– Biomicroscopy and ophthalmoscopy of the ocular structures *in vivo* enable changes in ocular pathology to be assessed which can mostly be documented by photography.
– In addition, the neural structures of the eye can be evaluated by electrophysiological measurements, such as visually evoked potentials (VEP) and electroretinography (ERG).
– Finally, there is the very valuable advantage of comparing the findings of a treated eye to the findings of the untreated eye in the same patient, thus providing clear-cut treatment results while, at the same time, sparing one eye to avoid potential adverse effects that would affect the entire visual system.

1.3.2 Specific Aspects of Emerging Ocular Treatment Options in Stem Cell Disease

Stem cell-driven treatments have not yet been clinically established in **lid pathologies**, while in **conjunctival disease** there are several clinical procedures that transfer stem cell populations from other parts of the non-affected conjunctiva (or from the contralateral eye) to the diseased part of the conjunctiva (conjunctival autograft). Another option is the transfer of intact mucous membrane grafting from oral or nasal tissues in severe cases of cicatrizing conjunctival disease in order to provide working stem cells. Yet another possibility, which has been performed for many years, is the transplantation of amniotic membrane. This supports the diseased conjunctiva with growth factors and acts as an organic protection for tissue defects. More recent therapeutic steps replace pathological conjunctiva with bioengineered synthetic material (acellular polymer porous glycosaminoglycan) which is integrated into neighboring healthy conjunctiva.

The **cornea** has been a target of stem cell treatment for many years due to the fact that corneal blindness is a severe and difficult-to-treat condition and a major cause of blindness in third world countries. Limbal stem cell insufficiency (LSCI) can be treated by transplanting sectorial allografts or autografts. The best result is achieved when part of the sectorial limbus is taken from the sound eye in unilateral LSCI. Limbal grafting is often the precondition for subsequent perforating keratoplasty to improve the outcome of transplant survival of the corneal donor tissue. In cases of severe pannus formation (vascularized fibrotic tissue covering the corneal surface and intruding into the upper corneal layers), keratoepitheliectomy, followed by amniotic membrane transplantation, may be another option, but lasting effects usually involve the replacement of the defective limbal tissue.

There are currently no established clinical procedures when it comes to stem cell dysfunction of the **trabecular meshwork**. Animal experiments attempt to improve the outflow parameters of the trabecular meshwork in induced glaucoma. Several sources of endogenous stem cells have been identified in the eye, some of which may be able to repair the damaged trabecular meshwork and restore functional regulation of aqueous outflow.

Initial stem cell treatments have been clinically performed in the **outer retina**, which harbors the retinal pigment epithelium and the photoreceptors. Despite hundreds of clinical trials, only one stem cell treatment has been approved for the U.S. market. Additional treatments that are nearing clinical acceptance use bone marrow mesenchymal stem cells to treat

inflammatory and immune-related conditions. Experimental injection of bone marrow stem cells into the vitreous body have been shown to slow down the process of deterioration of the visual field/visual acuity in selected patients. In rat models, differentiated retinal pigment epithelium (RPE) from human embryonic stem cells (hESCs) or human-induced pluripotent stem cells (hiPSCs) was transplanted into the subretinal space. Once they have been differentiated, cells from either source of PSC resembled mature RPE in terms of their morphology and gene expression profile. Following transplantation, both hESC- and hiPSC-derived cells maintained the expression of specific RPE markers. Recently, it was shown that multimodal delivery of isogenic mesenchymal stem cells yields synergistic protection from retinal degeneration and vision loss in rats. Valuable safety and efficacy data is starting to emerge from trials looking at cell therapies for age-related macular degeneration, Stargardt's macular dystrophy, retinitis pigmentosa, and ischemic retinopathies.

The **inner retina** is formed by the axons leading to the formation of the optic nerve. Neurodegeneration in glaucoma extends beyond the eye into the lateral geniculate nucleus and visual cortex. The disease even shares some characteristics with other degenerative disorders of the central nervous system. The treatment of optic atrophy (glaucomatous and other pathogenesis) consists of neuroprotective measures, like antioxidants, neurotrophic factors and Rho-pathway inhibitors. Neural and mesenchymal stem cells secrete growth factors which provide neuroprotective effects, reducing loss of retinal ganglion cells (RGCs) in animal models. Dental pulp stem cells (DPSC) are neural crest-derived ectomesenchymal stem cells that can be isolated relatively easily and non-invasively from the dental pulp of extracted postnatal and adult teeth. Accumulating evidence suggests that DPSCs are a very promising form of cellular therapy for the central nervous system (CNS) and for retinal injury and disease. A new treatment trial in rats promotes the differentiation of retinal Müller cells into ganglion cells *in vivo* to treat glaucomatous optic atrophy.

In **orbital disease**, especially in Grave's orbitopathy, treatment mainly aims at reducing the inflammatory reaction of orbital tissue and at immunemodulatory trials. Interestingly, there are several reports of patients who had received stem cell treatment for other reasons (mainly leukemia and severe sickle cell disease) and who developed Grave's disease following a bone marrow transplant.

2. PAX6 Gene and Implications for Neuronal and Ocular Development

2.1 Characterization of the Gene

The paired box 6 (PAX6) gene (formerly called the aniridia type II gene AN2 or oculorhombin) encodes a homeobox and paired domain-containing protein PAX6 that binds DNA at two different binding sites and functions as a regulator of gene transcription. It is located on the short arm of chromosome 11 (11p13) and is a highly conserved gene with a lineage down to eucaryota. In fact, 95 % of the human PAX6 gene is identical to the PAX6 gene found in zebrafish (ancestors that diverged from human evolutionary development around 400 million years ago). It has been discovered that the gene product of the human PAX6 gene regulates a cascade of other genetic processes involved in the development of the eye and brain and is thus classified as a "master control gene" in ocular development. It is heavily involved in the signaling pathways that regulate the pluripotency of stem cells. In embryogenesis, expression

is first seen in the forebrain, hindbrain, head ectoderm and spinal cord, followed by later expression in the midbrain. Genomic organization of the PAX6 locus varies considerably among species, including the number and distribution of exons, cis-regulatory elements, and transcription start sites.

The 16 confirmed exons are numbered 0 through 13 with the additions of exon α located between exons 4 and 5, and the alternatively spliced exon 5a. Each promoter is associated with its own proximal exon, resulting in transcripts that are alternatively spliced in the 5′–untranslated region.

2.2 Developmental Influences of PAX6

Activity of this protein is the key to the development of neural tissues, particularly in the eye. In addition, it contributes to the development of certain neural and epidermal tissues as well as other homologous structures, usually derived from ectodermal tissues. The gene is regulated by multiple enhancers located up to hundreds of kilobases away from its locus.

Mutations in the gene or in the enhancer regions can cause ocular disorders such as aniridia and Peters anomaly. Experiments in mice demonstrate that a deficiency in PAX6 leads to a decrease in brain size, brain structure abnormality leading to autism, lack of iris formation and an altered cornea. Knockout experiments produced eyeless phenotypes, reinforcing the gene's role in eye development. In the brain, the protein is involved in the development of the specialized cells that process smell. As a transcription factor, PAX6 acts at the molecular level in the signaling and formation of the central nervous system. PAX6 serves as a regulator in the coordination and pattern formation that is required for differentiation and proliferation to take place, ensuring that the processes of neurogenesis and oculogenesis are coordinated and carried out successfully. Although many functions of PAX6 are known, the molecular mechanisms of these functions remain largely unresolved.

PAX6 plays a role in prenatal development and is referred to as a transcription factor (as an activator and as a repressor). This means that PAX6 is a protein that binds to specific DNA sequences and activates or represses the genetic information delivered to mRNA.

Nonsense mutations of PAX6 can lead to a condition called aniridia which is associated with brain, olfactory and pancreatic abnormalities. PAX6 protein function is highly conserved across bilaterian species. Mouse and human PAX6 have identical amino acid sequences. The following figure (Fig. 2) demonstrates the effect of PAX6 haploinsufficiency in species, from drosophila to humans.

2.3 Inborn Pathologies and Systemic Diseases in Adulthood Related to PAX6 Dysfunction

With reference to OMIM[1] and the KEGG[2] Disease database, the following inborn pathologies and diseases are associated with PAX6 deficiency:

Ocular pathologies

- Anterior segment dysgenesis of different severities
  - Aniridia

1 OMIM – Online Mendelian Inheritance in Man.

2 KEGG – Kyoto Encyclopedia of Genes and Genomes.

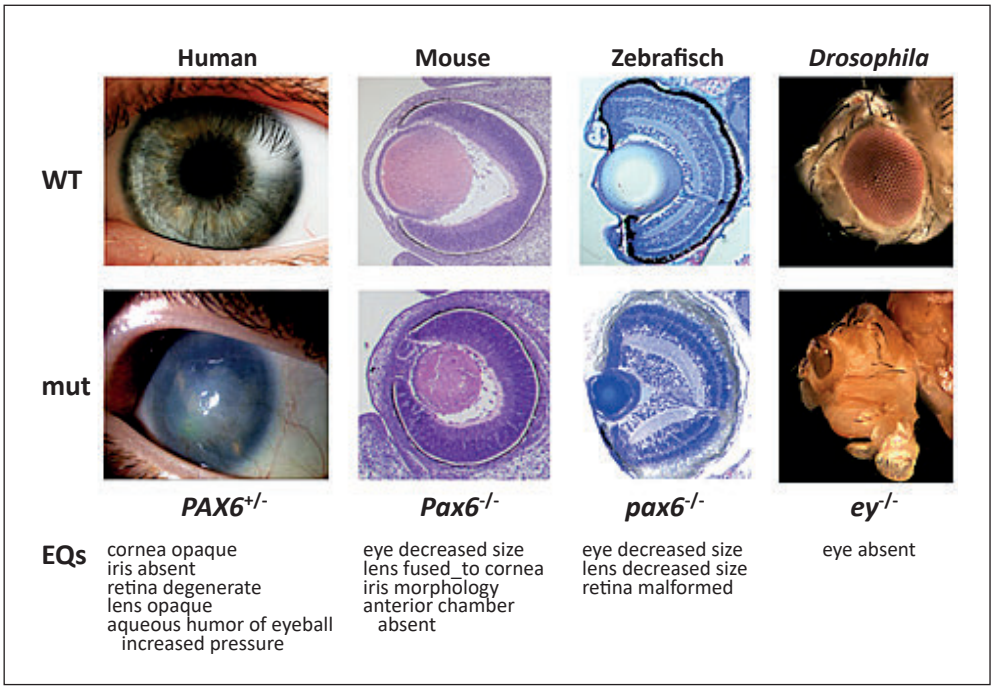

Fig. 2 Effect of PAX6 haploinsufficiency in different species. Figure from WASHINGTON et al. 2009

  - Peters anomaly, Peters plus syndrome: Peters anomaly plus systemic features
  - Anterior segment dysgenesis (ASD) including: anterior segment mesenchymal dysgenesis; Axenfeld anomaly
  - Autosomal dominant keratitis (ADK), hereditary keratitis
- Presenile cataract
- Ocular colobomata
  - Coloboma of the iris
  - Coloboma of the iris, choroid and retina
  - Coloboma of the optic nerve
  - Optic nerve pits (incomplete coloboma)
  - Morning glory syndrome (total optic nerve coloboma with staphyloma)
- Optic nerve hypoplasia
  - Bilateral optic nerve aplasia
- Foveal hypoplasia

Neurological pathologies

- Polymicrogyria
- Bilateral frontal polymicrogyria (BFP)
- Bilateral frontoparietal polymicrogyria

Systemic pathologies / syndromes
- WAGR syndrome: Wilms tumor, aniridia, genitourinary anomalies, mental retardation
- WAGRO syndrome: Wilms tumor, aniridia, genitourinary anomalies, mental retardation, obesity
- Gillespie syndrome: aniridia, cerebellar ataxia, mental retardation

Association to endocrine and metabolic disease
- Maturity onset diabetes type 1 (confirmed)
- Thyroid dysfunction
- Disturbances of diurnal rhythms (melatonin related)

3. Aniridia / PAX6-Syndrome

3.1 Clinical Picture

Congenital aniridia is a severe, pan-ocular congenital eye malformation that consists of changes in the anterior and posterior segments of the eye and occasional systemic findings (see above). Most cases are associated with dominantly inherited mutations or deletions of the PAX6 gene. Aniridia is a misnomer, as the name, coined by the first descriptions in the 18th century, neither reflects the pan-ocular signs nor the possible systemic manifestations. Aniridia is one of the rare causes of congenital low vision and may progress to blindness as a result of aniridia-related complications.

There are four aniridia-related complications that lead to the loss of visual acuity and permanent vision impairment:

- Presenile cataract formation,
- Glaucoma (with possible irreversible optic atrophy),
- Limbal stem cell insufficiency with corneal scar formation,
- Aniridia fibrosis syndrome: a profibrotic inflammatory (non-infective) reaction of the eye to intraocular surgery leading to fibrous scar formation and retinal detachment.

As the topic of the Leopoldina symposium was stem cell biology, emphasis will be placed on complication 3: loss of corneal transparency and ingrowth of vessels into the cornea due to limbal stem cell insufficiency (LSCI).

3.2 Anatomy of the Limbus and the Limbal Stem Cell Niche

Limbal stem cells provide the sustainability of corneal transparency, the integrity of corneal epithelium and surface homeostasis. They are located in the corneoscleral junction called the limbus and are responsible for the lifetime restoration and renewal of the epithelial tissue. Limbal stem cells initially transform to transient amplifying (TA) cells and then to cornea epithelium cells.

The epithelial stem cells reside in the limbal area in the so-called palisades of Vogt (PV). They are responsible for epithelial regeneration and for maintenance of the corneal-conjunctival border.

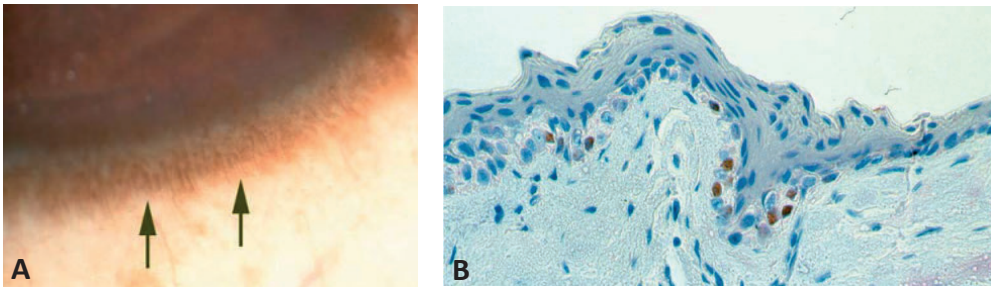

Fig. 3 Anatomy of the corneal limbal region. (*A*): Clinical aspect of the PV (arrows). (*B*): Histological section of the corneoscleroconjunctival area showing a section of a PV with immuno-stained limbal epithelial stem cells (brown). Figure adapted from Cursiefen et al. 2008.

3.3 Concept of the Limbal Stem Cell Niche

Human limbal epithelial stem cells reside in the basal layer of the epithelium (Ep) which undulates at the limbus. Daughter transient amplifying cells (TACs) divide and migrate towards the central cornea (arrow) to replenish the epithelium, which rests on Bowman's layer (BL). The stroma (St) of the limbal epithelial stem cell niche is populated with fibroblasts and melanocytes and has its own blood supply. Figure 4 shows the schematic anatomy of the limbal stem cell niche.

Aniridia/PAX6 syndrome is a disease mainly determined by the fate of limbal stem cells. It has the potential to be sight-threatening during life and is one of the diseases where stem cell research may yield improvements in patient care, vision and quality of life.

3.4 Aniridia-related Keratopathy (ARK) as Clinical Consequence of Limbal Stem Cell Insufficiency

Aniridia-related keratopathy (ARK) occurs in 20 – 90 % of patients. Corneal changes include recurrent erosions and ulcerations of corneal epithelium, tear film instability, dry eye, chronic pain, corneal vascularization, progressive corneal opacification and blindness. ARK is caused by a primary dysfunction of the limbal stem cells, probably by a limbal microenvironment alteration caused by the PAX6 gene mutation.

3.4.1 Pathogenic Basis of Aniridia-related Keratopathy (ARK)

Although ARK has been traditionally attributed to limbal stem cell deficiency, current evidence, based on clinical observations and animal models of aniridia, suggest that the proliferative potential of limbal stem cells may not initially be affected, and this corneal alteration may be related to an abnormality in the limbal stem cell microenvironment. Mutations in the PAX6 gene have been identified in a high proportion of patients with aniridia. Normal expression of the PAX6 gene is necessary for the normal development of the eye. This gene plays an important role in epithelial cell proliferation, migration and differentiation. The PAX6 gene is essential for cytokeratin-12 expression, a cytoskeleton protein restricted to the corneal epithelium and directly regulated by PAX6.

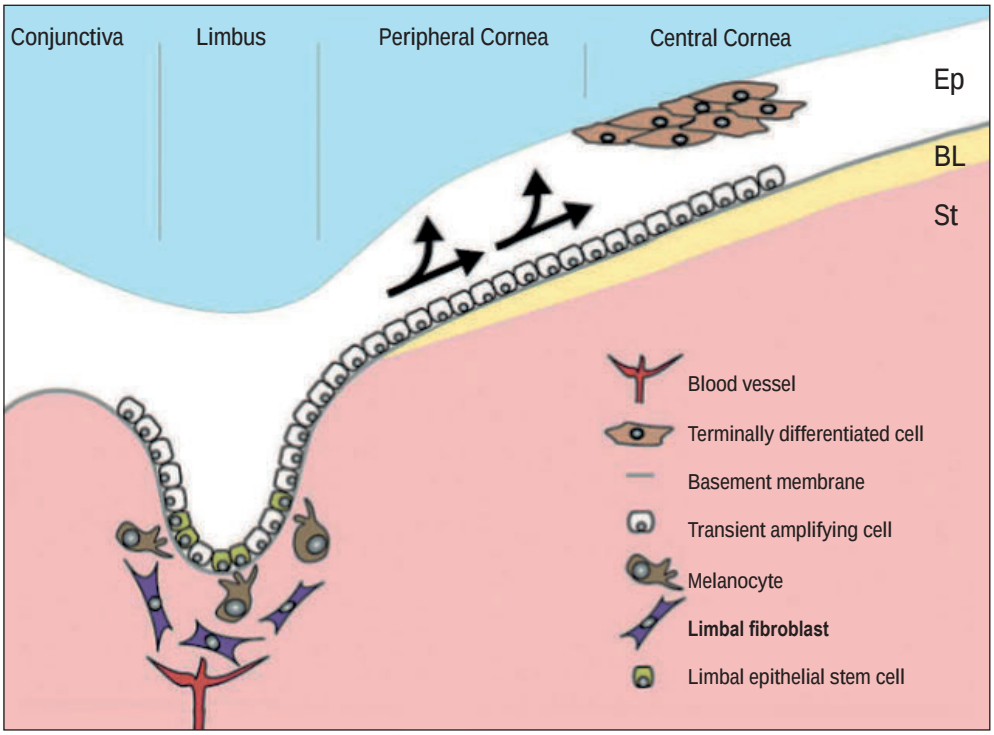

Fig. 4 Schematic anatomy of the limbal stem cell niche. Legend: Ep: epithelium. TAC: transient amplifying cells. Arrows: migration of TAC towards the central cornea. BL: Bowman's layer. St: stroma. With permission from Secker and Daniels 2009.

Reduced levels of cytokeratin 12 and 5 were found in PAX6 mutation. Keratins constitute the intermediate filaments of the epithelial cytoskeleton and their alteration is associated with epithelial cell fragility and disorders. These cytokeratins perform a vital role in cell-to-cell binding. PAX6 is essential for the expression of cell adhesion molecules like desmoglein and α and β catenin. These molecules are responsible for the maintenance of the cytoskeletal architecture, desmosome assembly, microtubule organization, the ability of cells to migrate in wound healing, and reinforcement of membrane attachments.

These alterations cause a fragile corneal epithelium that is clinically manifested by epithelial erosion and persistent epithelial defects. On the other hand, the PAX6 gene also contributes to the metabolism of the extracellular matrix. Matrix degradation is mediated by a group of enzymes known as matrix metalloproteinases (MMP). PAX6 regulates the MMP-9 or Gelatinase-B expression in the cornea. This enzyme is crucial for extracellular matrix remodulation in wound healing. The absence of MMP-9 causes a fibrin accumulation and inflammatory cell infiltration that is clinically manifested by a loss of corneal transparency and proliferative blood vessel stimulus. This situation generates a chronically wounded state. Moreover, the pattern of corneal innervations is modulated by PAX6 and the corneal nerves play an essential role in the maintenance of the ocular surface through the provision of neurotrophic support.

ARK is a progressive condition affecting the ocular surface of a great majority of patients with aniridia, although it does not always have visually significant consequences. Mutations in the PAX6 gene are thought to pre-dispose to LESC deficiency (LESCD) in aniridia. However, diagnosis of LESCD is challenging and mainly relies on clinical signs, including inflammation, vascularization, wound healing impairment, pain, reduction in visual acuity and infiltration of conjunctival goblet cells detected by impression cytology. This is because identification of LESCs in a normal eye is difficult in and of itself owing to a lack of definitive markers.

The onset of ARK can be accelerated by surgery (e.g. cataract removal or glaucoma filtration surgery) and exacerbated by factors such as dry eye. It can begin as early as the first decade of life, with peripheral vascularization gradually progressing to pan-corneal vascularization, keratinization and opacification. Interestingly, the limbal PV, which provide a niche for LESCs, cannot be detected by *in vivo* confocal microscopy in patients with severe ARK, suggesting a gradual loss of LESCs and/or their supporting environment as the disease progresses.

3.4.2 Loss of Corneal Transparency in ARK

The limbal epithelial stem cells (LESC) have self-renewal capabilities and, therefore, allow the corneo-scleral limbus to serve as a barrier. Multiple findings have led to the understanding that LESCs are located in the PV. PVs are radially oriented fibro-vascular structures located 1 – 2 mm from the limbo-corneal junction. They are more prominent in the upper and lower quadrants. Their morphology is believed to create an optimal microenvironment filled with stem cell nutrients and growth factors which regulate the process of cell division. Limbal epithelial stem cell deficiency (LESCD) inhibits ocular surface restoration.

ARK is clinically manifested as a primary limbal stem cell deficiency. Keratopathy, together with cataract and glaucoma, are the main causes of progressive visual loss in patients with aniridia, and it represents the main source of non-visual symptoms in these patients.

The cause of dry eye in aniridia is related to poor tear film quality produced by lipid layer dysfunction and corneal epithelial disorders. The lipid layer alteration is caused by meibomian gland dysfunctions, with stenosed atrophic meibomian orifices that change the lipid layer and facilitate tear evaporation. The severity of dry eye is related to the keratopathy grade.

The natural course of ARK progresses through several stages. Signs of keratopathy appear in the first decade of life with thickening of the peripheral corneal epithelium and without clinical manifestation. In the second decade, patients are afflicted with red eye and chronic irritation, and exhibit a thin and superficial vascularization in the peripheral cornea that gradually advances into the central cornea. Pain, photophobia and recurrent corneal epithelial erosions are common. In later stages, the keratopathy progresses until the entire cornea is involved, with a large increase in central corneal thickness. The central cornea is affected and there is a significant loss of vision due to subepithelial infiltrates, stromal opacifications and vascularization.

The opacification of the cornea in aniridia following repeated episodes of erosion and ulceration may be caused by a deficiency in matrix metallo-proteinase 9 (MMP-9), which is also regulated by the PAX6 gene. Matrix metallo-proteinases are responsible for the degradation of collagen during normal cell remodeling and wound healing. In PAX6 mutation in animal models, MMP-9 deficiency results in the accumulation of fibrin and the infiltration of

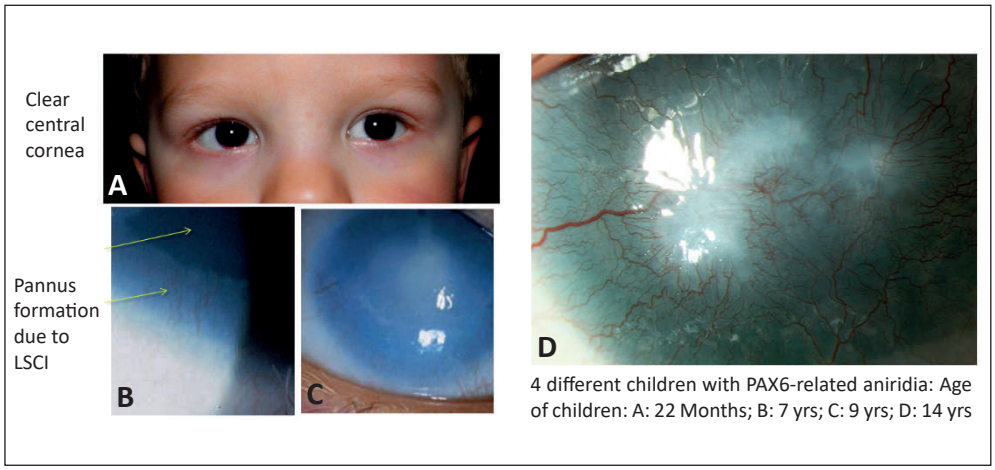

Fig. 5 Clinical aspect of limbal stem cell insufficiency in PAX6 haploinsufficiency and aniridia. Natural course of aniridic keratopathy. Thickening of the peripheral corneal epithelium in the first decade of life (*A*). Thin and superficial vascularization in the peripheral cornea in the second decade (*B*) that gradually advances into the central cornea (*C*). In later stages (*D*), the keratopathy progresses until the entire cornea is affected and there is a significant loss of vision due to subepithelial infiltrates, stromal opacifications and vascularization.

inflammatory cells. This disrupts the orderly arrangement of the collagen fibrils of the cornea and results in subsequent loss of transparency. The morphological changes in the cornea and limbus vary in ARK; *in vivo* confocal microscopy is a promising tool to determine the degree of LESCD in patients with ARK. The thickness of the central cornea is usually very increased (Lwigale 2015, Hill 2017, Naumann et al. 2008), frequently involving neovascularization, sub-epithelial fibrosis, changes in Bowman's layer and keratinization.

3.4.3 Formation of Corneal Vascularized Scars in ARK

Corneal transparency is essential for good vision and is an evolutionary, highly conserved sensory function. Since blood vessels within the cornea are incompatible with good visual acuity, the cornea of higher animals has developed strategies to protect the avascular cornea against the myriads of minor inflammatory and angiogenic stimuli that the cornea is constantly confronted with due to its exposed anatomical position.

Whereas the normal cornea is free of both blood and lymphatic vessels (so-called "corneal antiangiogenic privilege"), there is an intense network of both blood and clinically invisible lymphatic vessels at the limbus. This network extends circularly and effects a sharp transition into the avascular cornea. In the case of corneal inflammation, blood and lymphatic vessels start to grow into the cornea (pathologic corneal hem- and lymphangiogenesis). There seems to be a dynamic exchange between limbal macrophages and limbal lymphatic vessels, with macrophages being able to transdifferentiate into lymphatic vascular endothelial cells. This enables rapid outgrowths of lymphatic vessels in the case of severe corneal inflammation.

Damage to or dysfunction of the limbal SC population results in partial or total limbal SC deficiency, which has severe consequences for corneal wound healing and ocular surface integrity. Limbal SC deficiency is characterized by conjunctival epithelial ingrowth, vascu-

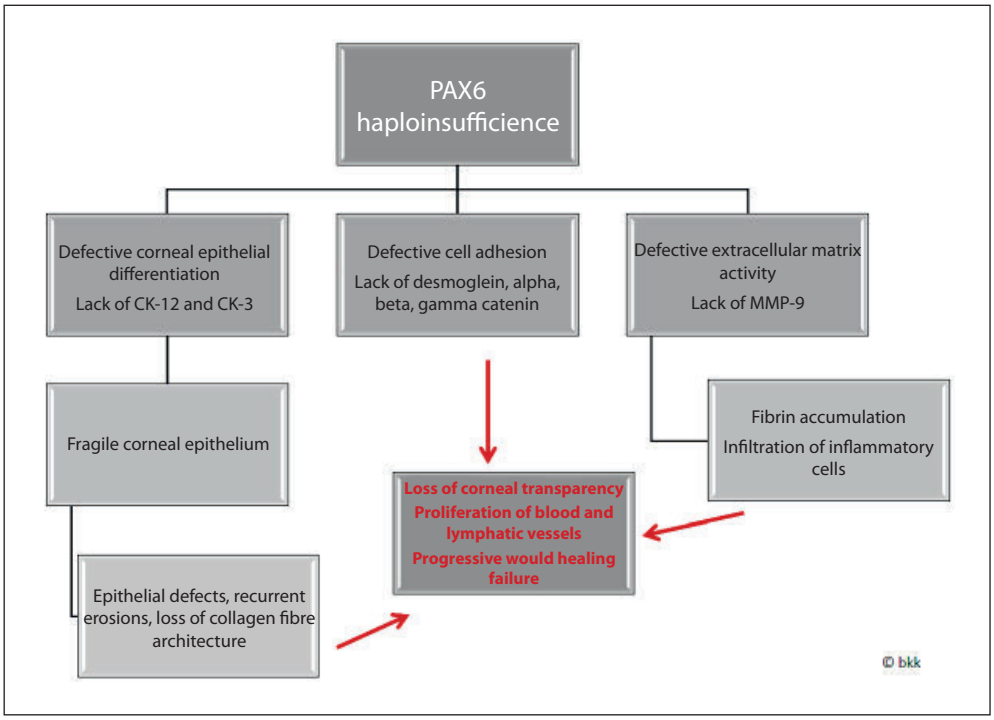

Fig. 6 Schematic Summary: PAX6 haploinsufficiency and pathological effects on corneal transparency

larization, chronic inflammation, recurrent erosions and persistent ulcers, destruction of the basement membrane, and fibrous tissue ingrowth leading to severe functional impairment.

Corneal antiangiogenic privilege is redundantly organized and already present very early during fetal development. In addition to antiangiogenic factors, the presence of soluble and fixed "decoy" receptors that bind and thus inactivate angiogenic growth factors, such as the vascular endothelial growth factor (VEGF), are an important mechanism that contribute to avascularity. The mechanisms of corneal antilymphangiogenic privilege, i.e. how the normal cornea stays free of lymphatic vessels, are still completely unknown. Aniridia-related keratopathy is a disease that combines limbal stem cell failure with defective corneal antiangiogenic privilege, as shown in Figure 7.

Different stages of ARK with corneal loss of transparency are associated with the pathological transgression of vessels across the limbus and centripetally into the cornea, demonstrated by anterior segment angiography.

3.5 Treatment Options in Limbal Stem Cell Insufficiency and Aniridia-related Keratopathy (ARK)

3.5.1 Non-surgical Treatment

In patients with slight keratopathy, treatment cycles with autologous serum (AS) lead to a subjective and objective improvement in the reduction of corneal erosion. In patients with

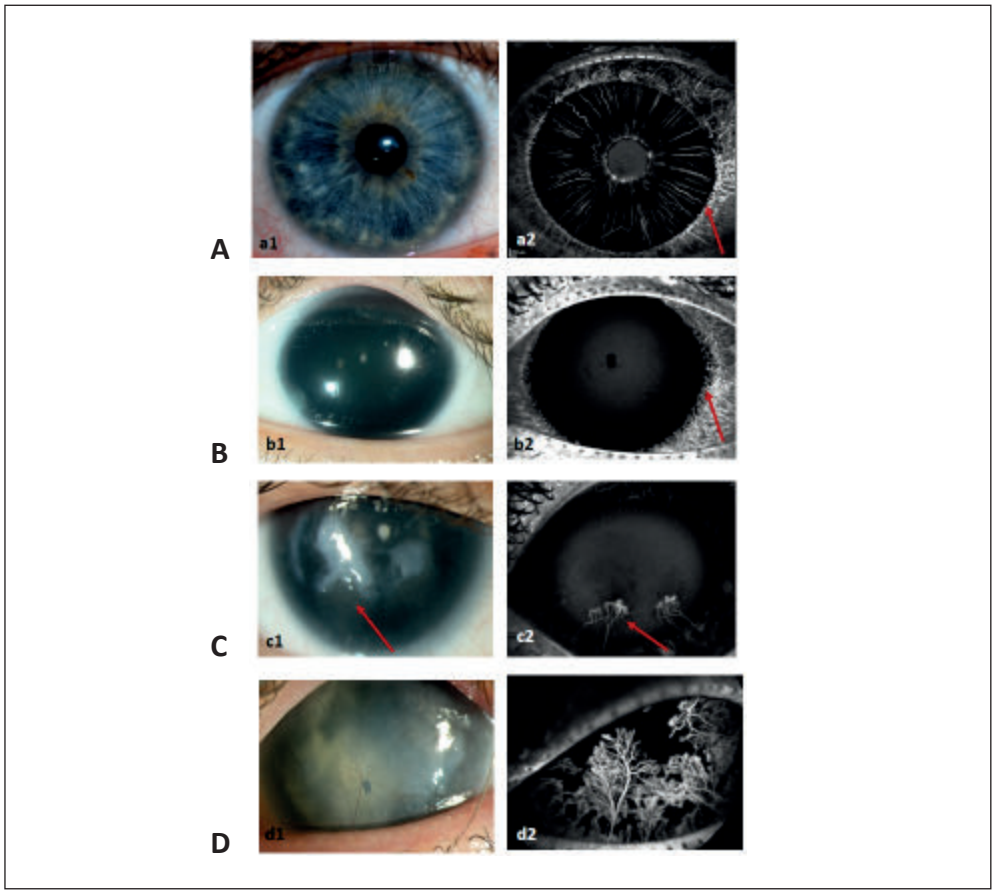

Fig. 7 Anterior segment angiography at different stages of ARK. Corneal loss of transparency is associated with pathological transgression of vessels across the limbus and centripetally into the cornea, shown on pictures and the angiographies of four patients. (*A*) Normal iris and respective angiography; (*B*) stage 1 ARK, broadening of limbal area; (*C*) stage 3 ARK, clear centripetal invasion of the vessels; (*D*) complete LSCI, stage 4 ARK, tree-like growth of neovasculature into the cornea.

moderate keratopathy (grade 2), treatment with artificial tears is not enough. In these patients, autologous serum or amniotic membrane transplantation (AMT) may be a useful (although temporary) measure to enhance the survival and expansion of limbal stem cells.

Treatment with AS has proven to be an efficient method for stimulating the stability of corneal and epithelial cells by supplying a number of growth factors (GF) which are scarce due to the ocular dryness associated with the majority of processes accompanying epithelization disorders. In patients with dry eye, AS provides some epitheliotrophic factors such as epidermal growth factor (EGF), basic fibroblast growth factor (βFGF), vitamin A, fibronectin, α2 macroglobulin, and neural growth factors that ease the proliferation, migration and adhesion of epithelial corneal cells. Because ARK is caused by a primary dysfunction of the limbal stem cell microenvironment, the epitheliotrophic factors presented in AS can help to treat the corneal changes that occur in these patients by improving the stem cell niche. AS significantly

improves Schirmer and BUT levels in patients with aniridia. Epithelial surface development, a better mucin expression and an improvement Meibomian dysfunction after AS treatment improve tear stability and, hence, BUT levels.

Treatment with AS greatly helps corneal epithelialization. Recurrent erosions are frequent complications in patients with ARK. This is caused by defective adhesion of the basal epithelial layers to the underlying basement membrane. It clinically manifests as repeated episodes of irritation, pain, epiphora and ocular hyperemia. A decrease in the erosion recurrence rate has been reported in patients with slight to moderate ARK that has been treated with AS.

3.5.2 Surgical Treatment

More severe cases of ARK require LESC transplantation to restore the ocular surface and vision. This has been successfully achieved using a combination of keratolimbal allografts and systemic immunosuppression. Meanwhile, the prospect of using a less invasive surgical technique (which may carry reduced risk for ARK patients) was achieved in 1997 when Pellegrini et al. described the first use of cultured autologous LESC therapy in two patients with corneal chemical burn injury. Since this landmark paper was published, a variety of culture methods and techniques for transplanting LESC onto the surface of the cornea have been attempted and reviewed elsewhere.

For patients with ARK, alternative sources of autologous stem cells could be beneficial since the use of systemic immunosuppression is not trivial and best avoided where possible. Cultured oral mucosal epithelial transplantation (COMET) has been used to treat patients with corneal chemical and thermal burn injury, Stevens-Johnson syndrome, mucous membrane pemphigoid (ocular cicatricial pemphigoid), and idiopathic ocular surface disorder.

Other types of cells that may be useful in restoring the ocular surface in ARK include hair follicle epithelial stem cells and olfactory cells. Alternatively, induced pluripotent stem cells (iPSCs) could play a future role in treating ARK. A protocol for generating corneal epithelial cells from adult dermal fibroblasts has been established. Theoretically, it should be possible to correct the PAX6 gene mutation in iPSC-derived corneal epithelial cells prior to transplantation back into the patient. This is perhaps one of the most exciting prospects.

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Cord Blood Stem Cells – A Dream for Future Medicine

Norbert Graf (Homburg/Saar)

Abstract

Cord blood can serve as a source for hematopoietic stem cell transplantation, a source for immunotherapy products and as a source for non-hematopoietic stem cells in the field of regenerative medicine. This article gives an overview about the use of cord blood stem cells.

Zusammenfassung

Nabelschnurblut stellt eine Quelle für hämatopoetische Stammzellen, für Immuntherapeutika und nicht-hämatopoetische Stammzellen in der regenerativen Medizin dar. Dieser Artikel gibt einen Überblick über die Verwendung von Stammzellen aus Nabelschnurblut.

1. Introduction

Cord blood is a unique cell product. More than 25,000 unrelated cord blood transplantations have been carried out up to now (Stavropoulos-Giokas et al. 2015).

Cord blood has several advantages over other stem cell sources. It is a waste product, and cells can be collected without harming the donor. There is a low risk of transmitting communicable diseases as well as a decrease in immune reactivity resulting in lower aGvHD[1] rates. Therefore, transplant products with 4/6 mismatched in HLA[2]-A, -B and DRB1 can be used. On the other hand, cord blood runs the risk of late engraftment as the number of nucleated cells are lower than in other sources. This causes a higher infection rate after transplantation. In addition, cord blood lacks donor lymphocytes that could be used in immune therapy.

2. Cord Blood Banks

Cord blood banks must provide safe, pure and potent products. In 2004 the Council of Europe's Committee of Ministers recommended cord blood banking based on cord blood donation and be used for allogeneic transplantation and for related research. Private cord blood banks exist alongside public banks.

1 aGvHD – acute Graft-versus-Host Disease.
2 HLA – Human Leukocyte Antigen.

According to Bone Marrow Donors Worldwide (BMDW) (http://www.bmdw.org/, 2016) 53 public cord blood registries are available in 36 countries worldwide. They have collected more than 680,000 cord blood units. In Germany only one public cord blood bank is active, served by the "Deutsche Knochenmarkspenderdatei") (DKMS) (http://parentsguidecord-blood.org/en/banks/dkms-cord-blood-bank, 2016). There are currently 17 public banks open in the United States that are funded by the government, various institutions and donations.

Private cord blood banks store cord blood for potential autologous or family member use only. Parents have to pay an up-front fee followed by a yearly rate. Private cord blood banks are a profitable business totaling US$ 4.5 billion in 2010 (Webb 2013). Many private banks advertise possible future uses for cord blood on websites that are not entirely supported by clinical evidence. This leads to parents feeling guilty that they may miss the unique chance of saving their child's life in the future. Private cord blood banks apply less stringent quality criteria than public ones (Stavropoulos-Giokas, et al. 2015). The number of privately stored cord blood units is unknown.

3. Cord Blood Banking

Various cells can be extracted from the umbilical cord. Cord blood contains hematopoietic stem cells (HSC) and unrestricted somatic stem cells (USSC). Mesenchymal stromal cells can be obtained from the Wharton's jelly umbilical cord (Roura et al. 2016). These cells can be isolated and banked to be used for treatment or research. There are also cord blood bank models that generate pluripotent stem cells (iPSC) from CD34 positive cells for further research.

Tab. 1 Clinical experience of cord blood stem cell transplantation in malignant and benign hematological malignancies. Adapted from Table 3 by Migliaxxio and Papayannopoulou 2015.

Source	No. of cases up to 2011	Disease treated	Advantages	Limitations	www.clinicaltrials.gov number
Single UCB	25,500	Benign hematological disorders and leukemias	Source of stem cells for patients who otherwise do not have a source 1 – 2 HLA mismatch tolerance	Cell dose: 2.5 –5.0 × 107 total nucleated cells/kg body weight This dose limits the use of single UCB to patients with a body weight of 20 – 40 kg (mainly children)	Not applicable
Double UCB	438	Hematological malignancies	Increased speed of engraftment Decreased relapse rates	Increased GvHD rates One UCP prevails	NCT00412360 randomized children with leukemia with single versus double UCB
Strategies to improve the stem/progenitor cells in the graft					
Co-culture with MSC	< 100	Hematological malignancies	Early neutrophil and platelet recovery	Double UCP transplantation in which the non-expanded unit prevails	NCT00498316

These cells can be provided to clinicians and industry (Stavropoulos-Giokas et al. 2015). Mesenchymal stem cells can be grown *in vitro* and differentiated into various cell types.

4. Clinical Trials Using Cord Blood Stem Cells

The first cord blood stem cell transplantation was done in Paris at the Saint-Louis Hospital by Prof. E. Gluckman in 1989 (Gluckman et al. 1989). A 6-year-old boy with Fanconi anemia received cryopreserved cord blood stem cells from his younger HLA-identical sister who was not affected by the disorder. Today 781 clinical trials can be found at www.clinicaltrial.gov by using the search term 'umbilical cord blood'. Adding the keyword 'transplantation' to the search reduces the number of trials to 324 (as of May 15, 2016). Most clinical trials using cord blood stem cells for transplantation are registered in the USA (229), followed by China (40) and Europe (33).

5. Hematopoietic Stem Cell Transplantation Using Cord Blood

Most of the trials deal with hematopoietic stem cell transplantation in malignant and benign hematopoietic diseases. The clinical experience is provided in Table 1.

6. Immunodeficiencies, Metabolic Diseases, Autoimmune Diseases

Further clinical trials are conducted for primary immunodeficiency syndromes, like severe combined immune deficiency (Morio et al. 2011) and Wiskott-Aldrich Syndrome (Knutsen and Wall 1999). Using cord blood stem cells enables reduced-intensity conditioning regimes with sustained engraftment. The GvHD rate is higher with Wiskott-Aldrich syndrome than with other primary immunodeficiency syndromes. There is no clear benefit over other sources of hematopoietic stem cells (Stavropoulos-Giokas et al. 2015).

In the case of metabolic diseases, hematopoietic stem cell transplantation plays a role in one lysosomal storage disease (Hurler syndrome). Over 500 patients with this disease have been treated until now (Stavropoulos-Giokas et al. 2015). Transplantation is able to preserve cognitive function due to the engraftment of donor microglial cells since they are derived from hematopoietic cells. Umbilical cord stem cells can be used as a source (Boelens et al. 2013). Hematopoietic stem cell transplantation has no value in other types of mucopolysaccharidoses (Stavropoulos-Giokas et al. 2015). Umbilical cord blood is extensively used for adrenoleukodystrophy and is beneficial if used at an early stage of the disease (Shapiro et al. 1995). Cord blood is not a good choice for osteopetrosis that can be cured by hematopoietic stem cell transplantation, since engraftment is problematic due to a low number of stem cells (Stavropoulos-Giokas et al. 2015).

Most of the clinical trials for autoimmune diseases have been conducted in China (Stavropoulos-Giokas et al. 2015). Umbilical cord blood transplantation is carried out for various autoimmune diseases like systemic lupus erythematosus, multiple sclerosis and primary Sjogren's syndrome. Further prospective clinical trials need to define potential benefits of such an approach (Stavropoulos-Giokas et al. 2015).

7. Cord Blood and Regenerative Medicine

As cord blood contains several subpopulations of non-hematopoietic cells, these cells can be isolated, grown and differentiated into various tissues. The treatment of diabetes, arthritis, burns, neurological disorders, myocardial infarction and other diseases using such cell products have been under discussion. Most of the work in this field of medicine is done in laboratories and has been carried out in animal models. Clinical trials have yet to yield definitive results (Stavropoulos-Giokas et al. 2015). Nevertheless, Sun et al. stated that "cord blood is an attractive source of multipotent stem and progenitor cells, which can serve as cellular therapeutic factories for many of these diseases" (Sun and Kurtzberg 2015).

8. Outlook

It seems appropriate to reconsider the potential future of cord blood biology, transplantation and banking. Increasing evidence shows cord blood contains pluripotent stem cells that have the potential to differentiate into non-hematopoietic tissue, such as cardiac, neurologic, pancreatic, and skin tissue. Extensive laboratory research is taking place to explore the potential therapeutic benefits of cord blood, also in regenerative medicine. The results of this research will be necessary to formulate future recommendations regarding autologous cord blood banking. Issues of patient consent, ethics and intellectual property will impact the use of these cells for commercial development (Stavropoulos-Giokas et al. 2015).

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Ergebnisse des Leopoldina-Förderprogramms IX

Stipendiaten der Leopoldina in den Jahren 2014–2015

Nova Acta Leopoldina N. F. Supplementum Nr. *33*
Herausgegeben von Gunnar Berg (Halle/Saale), Andreas Clausing (Halle/Saale) und Jörg Hacker (Halle/Saale)
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Das Leopoldina-Förderprogramm ermöglicht Postdoktoranden einen mehrjährigen Aufenthalt an einem internationalen Forschungsschwerpunkt ihrer Wahl. Damit wird der Wissenschaftlernachwuchs durch interessante Projekte in ausländischen Arbeitsgruppen der Spitzenforschung qualifiziert und auf spätere Aufgaben im deutschen Wissenschaftssystem vorbereitet. Der Band verdeutlicht die Vielfalt der vertretenen Fächer und Problemstellungen und berichtet über erfolgreiche Projekte. Die referierte weitere berufliche Entwicklung der ehemaligen Stipendiatinnen und Stipendiaten zeigt die Bedeutung der Fördermaßnahme und der im Studienaufenthalt erzielten Ergebnisse für eine weitere Karriere im Wissenschaftsbereich auf.

Wissenschaftliche Verlagsgesellschaft Stuttgart

Corneal Epithelial Stem Cells: Clinical Relevance and Novel Therapeutic Concepts for Reconstruction of the Ocular Surface (Abstract)

Daniel Meller (Jena)

The transparency of the cornea is essential for unimpaired vision, which partly depends on a functional corneal epithelium. To maintain functionality of the corneal epithelium, this tissue layer must be constantly renewed. Limbal epithelial stem and progenitor cells (LESCs), located at the basal limbal region of the cornea, regenerate the epithelium. Various ocular surface diseases are caused by the loss of LESCs or the dysfunction of the limbal niche. To treat limbal stem cell deficiency (LSCD) it is necessary to restore the regenerative system of the corneal epithelium. In addition to conventional transplantation of autologous or allogeneic limbal tissue, recent advances in tissue engineering have led to the development of new culture and expansion techniques of human LESCs which provide a new strategy to successfully treat LSCD. A transplant-ready epithelium can be created from a small autologous limbal biopsy with a limited amount of LESCs. This technique is popular since it expands cells from just a small biopsy, minimizing trauma to the donor eye. Of the expansion protocols for LESCs, co-culture with mouse 3T3 fibroblasts and expansion on carrier systems like fibrin or human amniotic membrane (AM) are being applied to patients. Autologous grafting of cultured limbal epithelium has led, in most of the treated cases, to a successful reconstruction of the corneal surface. Recently, a culture system for limbal epithelial cells was approved by the European Medical Agency (Holoclar) and has been granted marketing authorization. A review has been conducted of the challenges and controversies associated with these stem cell culture techniques for ocular surface reconstruction.

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Phänotypisierung – vom Schein zum Sein

Gemeinsames Symposium der
Österreichischen Akademie der Wissenschaften (ÖAW), der Deutschen Akademie der Naturforscher Leopoldina – Nationale Akademie der Wissenschaften und der Veterinärmedizinischen Universität Wien

am 19. und 20. März 2015 in Wien

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Der Phänotyp ist in Tierzucht und Genetik die erscheinende Gestalt. Ursprünglich als das äußere Erscheinungsbild, also die Summe aller äußerlich feststellbaren Merkmale und Eigenschaften eines Individuums, gefasst, kamen später auch die Lage und Größe innerer Organe, Verhaltensmerkmale und serologische Werte zum Phänotyp hinzu – obwohl sie ja nicht unmittelbar sichtbar sind. Phänotypisierung ist die spezifische, objektivierbare, standardisierte und systematische Beschreibung, Erfassung und Messung von Bildern der Merkmale, Strukturen, Funktionen und Krankheiten sowie deren grafische Auswertung und/oder quantitative Analyse. Phänotypisierung erfasst und misst unsere Bilder von der Wirklichkeit. Behandelt werden die Grundlagen der wichtigen bildgebenden Verfahren sowie verschiedene Formen von Phänotypisierung in Biologie (Morphologie, Genetik), Tiermedizin und Tierzucht. Dabei geht es auch um Fragen von Schein und Sein auf philosophischer Ebene.

Wissenschaftliche Verlagsgesellschaft Stuttgart

Wahrnehmen und Steuern
Sensorsysteme in Biologie und Technik

Vorträge anlässlich der Jahresversammlung
vom 19. bis 21. September 2014 in Rostock

Nova Acta Leopoldina N. F. Bd. *122*, Nr. 410
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Gottfried Schmalz (Regensburg) und Eberhart Zrenner (Tübingen)
(2015, 283 Seiten, 131 Abbildungen, 3 Tabellen, 29,95 Euro,
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Mensch und Tier sind für ihr Überleben in der Auseinandersetzung mit der sie umgebenden Umwelt auf die Wahrnehmung optischer, akustischer, olfaktorischer, gustatorischer und haptischer Eindrücke angewiesen. Mit ihren sensorischen Systemen können sie die vielfältigen chemischen und physikalischen Reize aufnehmen, die der Organismus verarbeitet und die schließlich das Verhalten und die Gefühlswelt beeinflussen. Für die Problematik der Sensorik und des Wahrnehmens des Menschen spielt die Begrenzung durch entsprechende Einschränkungen eine besondere Rolle. Diese kann durch die wissenschaftlichen und technischen Möglichkeiten über künstliche Sensorsysteme immer besser kompensiert werden. Schließlich kann sich wahrnehmendes Steuern vom Menschen gänzlich lösen und z. B. Robotern zugewiesen werden. Die Verbindung der Sinn- und Wahrnehmungsproblematik mit ästhetischen Fragestellungen und künstlerischen Herangehensweisen liefert ein weiteres interessantes Diskussionsfeld. Der Band behandelt auch die Themen „Biologische Kommunikation“, „Hören und Sehen“, „Sprache, Denken und Lernen“, „Medizintechnik, angewandte Biomechanik und Robotik“ sowie „Gesellschaft“.

Wissenschaftliche Verlagsgesellschaft Stuttgart

Geschlechtsabhängige Vererbung – mehr als Gender und Sex

Gemeinsames Symposium der
Deutschen Akademie der Naturforscher Leopoldina – Nationale Akademie der Wissenschaften, der Österreichischen Akademie der Wissenschaften (ÖAW) und der Veterinärmedizinischen Universität Wien

am 27. und 28. März 2014 in Wien

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Geschlechtsabhängige Vererbung folgt nicht den Mendelschen Regeln und ist vom Geschlecht der Eltern abhängig. Sie trifft daher als wichtiger Sonderfall in der biologischen Forschung auf besonderes Interesse. Ausgehend von der biologischen und sprachlichen Differenzierung von Genus, Geschlecht, Sex und Gender werden in den Beiträgen Formen der X-chromosomalen Vererbung, die extrachromosomale mitochondriale Vererbung und geschlechtsbegrenzte Erbgänge analysiert. Im Fokus stehen sowohl traditionelle Versuchstiere als auch landwirtschaftliche Nutztiere. Ein besonderes Kapitel ist der geschlechtsabhängigen Epigenetik vorbehalten. Fragen der Rinder-, Pferde- und Hundezucht werden im Gesamtkontext ausgehend von praktischen Fragen bis auf die Ebene der Molekularbiologie diskutiert.

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