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Highlights

The body's integrated repair kit: Studying mesenchymal stem cells for better ligament repair

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ABSTRACT

In this issue of the *Biomedical Journal*, we learn that the sport injury-prone knee ligaments might harbour their own repair kit in the form of mesenchymal stem cells, and that TERT transformation helps to keep these cells longer in culture for more extensive studies. In addition, we get a demonstration that diffusion tensor imaging can reliably show the activity of specific neural circuits, that rheumatoid arthritis patients are more prone to insulin resistance, and that platelet-enriched plasma gels significantly improve wound healing after pilonidal sinus surgery. Furthermore, two procreation-related articles inform us that growth hormone treatment improves endometrial receptivity in older women, and that elevated maternal liver enzymes do not impact on the outcome of laser therapy for twin–twin transfusion syndrome. Finally, our attention is brought to the importance of subjective well-being evaluation for orthodontic correction needs, as well as the possibility that exercise could maybe increase sperm telomere length.

Spotlight on original articles

The body's integrated repair kit: studying mesenchymal stem cells for better ligament repair

A few frog hearts beating for a few hours in a salt solution represented the very first steps towards the establishment of cell culture at the end of the 19th century [1]. By now this is an everyday feature in the work of many biologists, yet at the time it was revolution regarding our access to the clockwork of living beings. Progressively, culture media improved, allowing for longer survival and proliferation. About half a century later, another milestone was achieved in the form of the first immortal cell lines, with the iconic HeLa human

cancer line at the forefront [2]. For a long time, only cancer cell lines, or cells immortalised via oncogene expression, could be kept in culture indefinitely. The next breakthrough happened in 1998, when James Thomson succeeded in deriving and culturing the first human embryonic stem cells (ESC) from the inner cell mass (ICM) of blastocyst stage embryos [3]. Not only did this provide researchers with an immortal cell line with way more physiological properties than cancer cells, but also unprecedented possibilities to study cell fate commitment, as ESCs can be differentiated into virtually any cell type of the three embryonic germ layers. Additionally, it sparked massive hopes for regenerative therapy – the replacement of lost or diseased tissue by *in vitro* engineered cells and organs [4] [Fig. 1].

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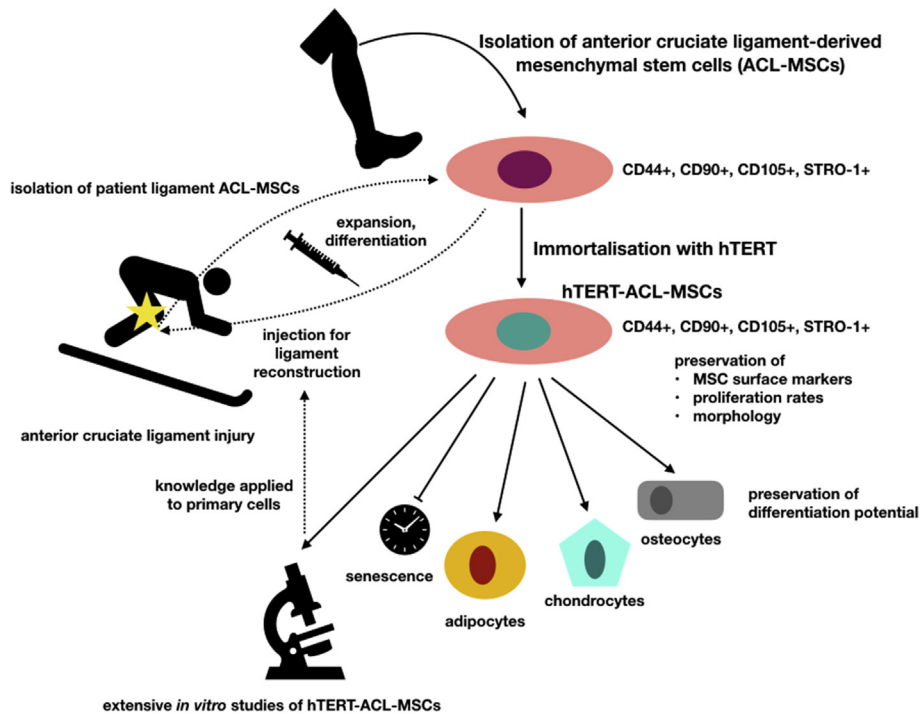


Fig. 1 hTERT-immortalisation of anterior cruciate ligament derived mesenchymal stem cells (hTERT-ACL-MSCs) improves research conditions on their therapeutic potential. Mesenchymal stem cells are praised for their therapeutic potential and also found in the ACL. However, they are not easy to obtain and enter senescence after several passages in culture. Immortalisation of ACL-MSCs with hTERT does not alter their MSC properties nor their differentiation potential but prevents them from entering senescence. Thus, hTERT-ACL-MSCs could be a very useful tool to further study these cells, and optimise their culture and differentiation conditions in order to eventually use primary ACL-MSCs for ligament reconstruction in the future.

The excitement peaked in 2006 with the discovery that terminally differentiated cells such as fibroblasts could be reprogrammed to an ESC-like stage by the expression of only four pluripotency key transcription factors by Shinya Yamanaka [5]. These induced pluripotent cells (iPSCs) yielded the 2012 Nobel prize for Yamanaka, and gave regenerative therapy without doubt its greatest boost so far. They hold the potential for autologous tissue replacement, in addition to making patient-specific disease models available in a dish [6], including “mini-brains” with the recent 3D organoid culture systems [7]. To date, the first clinical trials for stem cell therapies have taken place and produced some promising results, namely in retinal cell replacement [8].

Beyond doubt, pluripotent stem cells are incredibly powerful, and inevitably, there is a dark side of the force. True pluripotency, in the physiological setting of the developing organism, is an extremely ephemeral stage. Maintaining this property artificially in culture requires very “repressive” medium compositions and careful manipulation, as any stimulus tends to trigger differentiation. The organism has its reasons to minimise pluripotency though, because these cells share a disconcerting amount of properties with cancer cells, such as immortality and high proliferation rates. Thus, it does not come as a surprise, that multiple tumour types hijack stemness signalling pathways, and that a dedifferentiated tumour phenotype correlates with bad prognosis [9]. Therefore ESC- or

iPSC-based regenerative therapy has therefore to meticulously make sure that no undifferentiated cells that could go rogue remain in engineered cell mixture. For these reasons, along with the incompatibility of most reprogramming methods with patient safety, a special progeny of the original pluripotent stem cell pool is receiving increasing attention. Adult stem cells (ASCs) are defined as small populations of tissue-specific, undifferentiated, self-renewing (but not immortal) cells with the potential to give rise to all cell types of a given organ – this property is termed multipotency. The idea of harvesting, expanding and directing their differentiation for tissue regeneration has given rise to multiple trials, including for myocardial repair, blood disorder therapy, and battling diabetes [10]. Nonetheless, the exact definition and characterisation of ASCs has been subject to much controversy. It is still debated if ASCs are better described by phenotype, such as a certain combination of cell surface markers, or rather by functional potential, which depends in turn of the cellular microenvironment [11]. For example, although they are certainly the best known and studied model of ASCs, the hierarchical model of haematopoietic stem cell (HSCs) stepwise differentiation into all blood lineages has been recently challenged by a revised model, advocating for heterogeneity and flexibility among the HSC and progenitor populations [12].

The other most eminent type of ASCs is the rather heterogeneous class of mesenchymal stem cells (MSCs). They

reside in many tissues, including muscles, bone marrow and adipose tissue, and can differentiate into osteoblasts, chondrocytes, and adipocytes. Furthermore, they display not yet fully understood immunomodulatory properties. Ongoing phase II trials comprise the treatment of a wide range of diseases, from myocardial infection to Crohn's disease [13]. Yet the most spectacular success of MSC-based therapy was obtained this year in one case of spinal cord injury treatment by the injection of autologous adipose tissue-derived MSCs, allowing the patient to regain substantial motor and sensory functions [14].

In this issue of the Biomedical Journal, the group of Andre Steinert focuses on a special requirement of regenerative treatment by MSCs [15]. Rupture, or other damages to the anterior cruciate ligament (ACL) of the knee are very frequent sport injuries, both in professional athletes and the general population. Footballers, skiers and gymnasts represent the top three disciplines at risk, and women are globally more prone to ACL damage than men [16]. Without surgical repair, the articulation stays unstable and susceptible to more damage, thus severely limiting the patient's activities and quality of life. The dominant surgical repair method consists in replacing or complementing the torn ligament with an autologous tendon graft. However, the outcome of this type of surgery is not always satisfying. Failed reconstruction or the development of osteoarthritis are possible complications [17].

The group had previously shown that a type of MSCs, complying with the requirements of surface markers and differentiation potential, can be isolated from the ACLs of young donors [18], opening new therapeutic possibilities. Nonetheless, one downside of multipotent stem cells is that they are not immortal, and enter senescence after a certain amount of passages in culture. In order to perform more studies *in vitro* on ACL-derived MSCs without being restricted by limited lifespan and variability between donors, Prager et al. suggest the immortalisation of the primary cells using the human telomerase reverse transcriptase (hTERT). hTERT, the catalytic subunit of telomerase, is considered an oncogene, as its abilities to extend telomeres and block cell death promote tumour aggressiveness and metastatic potential [19]. Nevertheless, it is a useful tool to preserve mortal cell lines for more extensive studies without further changing their properties, as it has been already done for muscle cells [20], and preadipocytes [21].

The authors make it indeed very clear that their model is not meant for any application in patients but uniquely for better studies in cell culture. Though one could eventually imagine an application in animal models.

After successful transformation of two freshly derived primary ACL-MSCs, they demonstrate that hTERT-ACL-MSCs do not display any morphological or phenotypical changes that would be indicative of cell identity loss and cancerous transformation. Therefore, the cells exhibit no changes in proliferation rates and preserve the morphology and cell surface marker expression profile characteristic of primary, early passage MSCs derived from the same source. Moreover, they retain their differentiation potential, demonstrated by chondrogenic, osteogenic and adipogenic differentiation assays. Additionally, hTERT-ACL-MSCs showed significantly reduced signs of senescence after long-term

culture. Together, these results indicate that hTERT transformation of ACL-MSCs is a suitable method to render the cells available for more and longer *in vitro* studies without altering their essential properties.

Furthermore, the authors briefly touch upon the subject of age differences between the MSC donors. Evidence points towards the exhaustion of ASC pools during aging, in a complex entanglement of cause and effect [22,23], meaning that it might be harder to obtain autologous MSC material from older patients. This point requires further studies and larger sample sizes though, in order to determine ultimately how realistic MSC treatment for ligament injuries will be in the long run.

Also in this issue

Original articles

Diffusion tensor imaging allows for reliable imaging of the baroreflex neural circuit

Seeing the brain in action is by itself utterly fascinating. Several neuroimaging techniques, such as functional magnetic resonance imaging (fMRI) or positron emission tomography have rendered this possible, and sparked in 2010 the "Human Connectome Project" (HCP), a 40 million dollar project aiming at a thorough mapping of the structure, connectivity and functional compartmentalisation of the human brain [24].

The complete discernment of a particular neural process, however, requires simultaneous information regarding the neural network structures (tractography) and changes in neural activity. Diffusion tensor imaging (DTI) can achieve both, assert Tsai et al. [25]. Based on the anisotropy of water diffusion, DTI allows for the visualisation of white matter structure, as well as for the detection of electric variations corresponding to action potentials [26]. As a proof of principle, Tsai et al. demonstrate in their present study that DTI can detect baroreflex functionality [27]. The latter is a homeostatic mechanism ensuring the maintenance of blood pressure via a very fast negative feedback loop linking neurons in the aortic arch and carotid sinuses with autonomic nervous centres in the brain stem [28], a challenging region of the brain to image. The authors succeed in robustly detecting the neuronal traffic corresponding to the baroreflex circuitry in the rat and uncovered that it does not necessarily require a connection between the nucleus tractus solitarius (NTS) and caudal ventrolateral medulla (CVLM). Moreover, using a mevinphos intoxication model for brain stem death, they show that reversible versus irreversible disruption of the NTS and rostral ventrolateral medulla (RVLM) predict survival or death of the animal.

Is the atherosclerotic risk in rheumatoid arthritis mediated by insulin resistance?

Rheumatoid arthritis (RA) is a systemic autoimmune inflammatory disease which is mainly characterised by inflammation and thickening of the joint capsule. The causes are partly genetic and partly environmental, and lead to the production of autoantibodies such as rheumatoid factors and antibodies against citrullinated proteins. Persistent pain, deformity and

disability are the debilitating consequences [29]. Moreover, RA correlates strongly with an increased risk for cardiovascular disease and mortality, mainly due to a higher incidence of atherosclerosis [journal:wp]. Both diseases share common risk factors and actors, notably proinflammatory cytokines. However, the picture might lack intermediate elements, such as insulin resistance induced by the increased secretion of cytokines and adipokines.

Hence, Guin et al. attempt to assess the link between inflammation, insulin resistance, and atherosclerotic risk in RA patients [30]. Their analysis demonstrates that RA patients have a significantly higher probability of developing insulin resistance compared to the general population. As a consequence, conventional drugs used against RA might be insufficient to control TNF- α -mediated inflammation.

Platelet-rich plasma gel accelerates wound healing after pilonidal sinus surgery

Both the 14th century “knight’s bottom” and WWII “jeep disease” refer most likely to the same symptom: pilonidal sinus disease (PSD), literally a “nest of hair” [31]. If there is a list of slightly embarrassing ailments, PSD has good prospects of a top position, as it consists in the formation of an abscess, often filled with hair, debris and granulation tissue, near the coccyx [32]. Yet it causes major discomfort, and is rather frequent, affecting 0,7% of the population, and occurs mostly in hirsute males between puberty and 30 years [33]. Although the diagnosis is usually straightforward, the treatment has led to a surprising amount of controversies and nautical metaphors [31,34,35]. Treatment options range from filling the cyst with crystallised phenol [Adriat:vw] or fibrin glue [36] to various surgical approaches [37]. Some strategies involve the wide range excision of both cavity and sinus. Improving both wound healing quality and time are thus substantial for the patient to return to a normal lifestyle and avoiding recurrence.

Here, Mohamadi et al. examine the effectiveness of autologous platelet-rich plasma gel for wound healing after PSD surgery. They observe that individuals treated with plasma gel display significantly faster wound healing, correlated with better angiogenesis, as well as lower pain levels and antibiotics requirements [38], strongly backing their recommendation of this type of treatment after PSD surgery.

Growth hormone treatment improves endometrial features for pregnancy in older women

The average age at first birth has been on the rise since the 1970s in Western Europe, Japan, and the United States [39]. Although some studies claim a connection between life expectancy extension and late births [40], advanced maternal age correlates with an increase in adverse pregnancy outcomes [41] and certain birth defects due to chromosomal abnormalities, mainly trisomies, due to a decrease in chromosomal cohesion in aging oocytes [42].

Assisted reproductive technology (ART) has further expanded the possibility of late pregnancies, but also amplified the need for therapies to improve their outcomes [43], which range from yoga [44] to intense hormonal treatments [45]. The main medical uses of Growth Hormone (GH), also known as somatotropin, are replacement therapies for

individuals with a GH deficiency [46], or to treat GH-independent short stature conditions [research:vc]. However, GH supplements have proven to ameliorate pregnancy and live-birth rates, an effect thought to be mediated by improved oocyte quality [47].

Lan et al. hypothesize that GH might additionally enhance implantation and receptivity through beneficial effects on the endometrium. In their present study, they review the effect of GH supplements on the outcomes of *in vitro* fertilisation and intracytoplasmic sperm injection embryo transfer in women aged over 40 years. In line with their speculation, the authors note that GH treatment led to a significant reduction in cycle cancellation rates, and an increase in favourable ultrasonic endometrial patterns correlating with better implantation and pregnancy rates [48].

Elevation of maternal liver enzymes is frequent in twin–twin transfusion syndrome but does not impact on laser therapy outcomes

Identical twins sharing the same placenta (monochorionic) happen in/account for 0,7% of all pregnancies. In 5–10% of these cases, twin-to-twin transfusion syndrome (TTTS) can take place, where the twins’ blood supplies become connected [49]. A disproportionate blood flow between the fetuses entails high morbidity and mortality rates, as the donor twin faces a decreased blood volume and growth retardation, while the recipient twin undergoes a blood overload, which can lead to heart failure [50]. Early detection and management are thus crucial. So far, the only therapy to stop the disease progress is laser therapy, namely the “Solomon technique” – a rather ironic term, given the story from the Hebrew Bible, where King Solomon, confronted by two women claiming both to be the mother of an infant, suggested to cut the baby in two [51]. The delicate surgical technique however consists in the selective foetoscopic laser photocoagulation of the vascular anastomoses between donor and recipient along the vascular equator from one side of the placenta to the other [52].

Having noted a TTTS case paralleled by elevated liver enzyme levels (ELEzs), Chang et al. undertook to systematically investigate the frequency of ELEzs in TTTS before laser therapy, as well as a potential association with the perinatal outcomes, given that ELEzs are known markers of liver injury and related pregnancy complications [53].

Their retrospective analysis reveals that 19,4% of TTTS patients display ELEzs before foetoscopic laser therapy, and that this feature does not correlate with any adverse peri- or postnatal consequences [54].

The importance of patient auto-evaluation before orthodontic and orthognathic correction

Orthodontic defects impact of course on the proper mechanical functioning of jaws and dentition, but have also a massive influence on aesthetics and the resulting self-perception and quality of life [55].

In this regard, the subjective evaluation of the treatment needs and its outcome by the patients themselves is more and more taken into account by the clinicians [56].

Here, Liu et al. sum up the results from several types of questionnaires submitted to control subjects, and patients receiving either orthodontic treatment or orthognathic

surgery prior to the onset of treatment [57]. While there were little to no differences between the groups regarding their answers to the SF-36 survey, which rather superficially covers general fitness and emotional wellbeing, patients upon treatment start had higher negative impacts in the oral-health specific OHIP-14 questionnaire. Namely class II malocclusion patients suffered the most from aesthetic discontent induced psychological stress, thus emphasizing the health-impact of defects not strictly restricted to physiological processes.

Brief communication

Exercise might promote sperm telomere lengthening in high responders

The older, the wiser is a common saying; much less known is the fact that older men not only possess more life experience, but apparently also longer sperm telomeres than their younger counterparts [58,59]. These protective caps at the ends of our chromosomes are known to erode during aging in somatic cells and thought to remain stable in the germline. The error might originate from the latter statement - unlike initially hypothesized though, this phenomenon does not seem to be due to a mysterious telomere elongation mechanism in sperm over the male lifetime, but instead to an intergenerational, continuous telomere shortening due to telomere erosion in the female germline, accelerated through the increase of maternal age at childbirth [60,61].

While no consensus exists yet if humanity is thus inevitably doomed, Joshua Denham has decided to explore if somatic telomeres could be salvaged by other means, such as exercise performance [62].

The preliminary results of this exploratory investigation suggest that, although there seems to be no correlation between sperm telomere length and cardiorespiratory fitness, high responders to physical exercise display telomere lengthening, whereas low responders experience shortening. The author encourages a more in-depth analysis on an increased sample size of these associations, and an investigation of the role of oxidative stress levels as their mediator.

Conflicts of interest

The author declares no conflict of interests.

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