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Stem Cell Research 2018-Chondrogenic Differentiation of Adipose-Derived Stem Cells by Radiofrequency Electric Stimulation- María Luisa Hernández-Bule- Ramón y Cajal University Hospital

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Introduction

There is ample evidence that stimulation with electric currents and electric and/or magnetic fields can induce a variety of cellular and molecular responses, including microfilament reorganization, redistribution of cell surface receptors or cell migration, as well as changes in intracellular calcium dynamics and in stem cell proliferation or differentiation. This body of evidence has provided indications that some electric and/or magnetic stimuli may exert favourable effects in the control of cell and tissue homeostasis, thus intervening in tissue repair and regeneration processes. Indeed, electrotherapy has been successfully applied to bone fracture consolidation, soft tissue regeneration, nerve fibre repair or treatment of cancerous lesions. Electric and electromagnetic therapies have proven also effective in the treatment of osteoarticular lesions such as osteoarthritis or degenerative disc disease. Similarly, capacitive-resistive electric transfer (CRET) electrothermal therapies, based on non-invasive application of radiofrequency (RF) electric currents, have been used successfully in regeneration of muscle, tendons and ligaments . As for cartilage and other tissues having poor capacity for regeneration and cellular self-renewal , although the potential repairing effects of electrotherapies remain a matter of debate, it has been reported that exposure to electric or electromagnetic stimulation can induce in articular chondrocytes cellular responses involved in prevention of degenerative damage. Evidence of this kind has served as a basis for proposing that stimulation with specific electric and/or magnetic parameters could favour cartilage regeneration through promotion of extracellular matrix protein synthesis and/or of chondrocyte or prechondrocyte proliferation. The mechanisms underlying these effects would involve electrical stimulation of cell membrane receptors which, through activation of signalling molecules, would trigger a cascade of effects resulting in cellular migration, proliferation or differentiation. In fact, evidence exists that stem cells present in the cartilaginous tissue could be a plausible target for treatment with electric fields or currents. Indeed, stimulation with 500 mV/mm, direct current electric field has been reported to promote survival of grafted neural stem cells, guiding their migration and stimulating their differentiation and functioning within the lesion [29]. Also, time-varying electric fields (60 kHz, 20 mV/ cm) and pulsed electromagnetic fields (27.1 MHz) have been reported to promote osteogenic differentiation of mesenchymal stem cells. Previous results by our group have shown that intermittent exposure to subthermal densities of RF (448 kHz) currents of the type applied in CRET therapies promotes proliferation in undifferentiated cultures of adipose-derived stem cells (ADSC) obtained from healthy donors [6]. Such proliferative response did not affect the subsequent ability of the ADSC to normally differentiate towards chondrocyte, adipocyte or osteocyte lineages when the cultures were supplemented with the corresponding differentiating factors. Our studies also revealed that in vitro exposure to the above subthermal RF currents can modulate the expression of genes controlling the synthesis and expression of proteins intervening in early stages of the adipogenic differentiation of ADSC. Based on the above experimental evidence, the possibility can be posed that CRET currents could also be effective in modulating processes intervening in cartilage regeneration, through stimulation of stem cells present in the damaged or degenerating tissue. Thus, the aim of the present study was to investigate the potential action of the in vitro electrostimulation with subthermal pulses of CRET current on early chondrogenic differentiation of ADSC. The cellular response was

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assessed by analysis of cell proliferation, quantification of extracellular matrix components synthesized during chondrogenesis, analysis of gene and protein expression of the chondrogenic markers L-Sox5 and Sox6, and assessment of the activation of the Mitogen-Activated Protein Kinase Extracellular Signal-Regulated Kinases 1 and 2 (MAPK ERK1/2) signaling pathway, which has proven an important regulator of cartilage-specific gene expression in a variety of chondroprogenitors and chondrogenic cell types

Material and Methods

Cell culture

ADSC were isolated from subcutaneous adipose tissue surgically obtained from 4 healthy donors: two men, 65 and 69 years old, and two women of 28 and 35. This protocol, which has been described in detail in previous studies [6], met the ethical standards applicable in the European Union, and was approved by the ethics committee for clinical trials of Hospital Universitario Ramón y Cajal. Briefly, ADSC were isolated from 0.5-1 cm3 pieces of fat and sliced into 1-2 mm3 fragments which were subsequently digested with 1 mg/ml collagenase A (Roche Applied Science, Basel, Switzerland) and centrifuged to isolate the vascular-stromal fraction. The resulting pellet was resuspended in culture medium (MesenPro-RSTM, Gibco, Invitrogen, Camarillo, CA, USA) supplemented with 1% glutamine (Gibco) and 1% penicillinstreptomycin (Gibco), and the cells were seeded in a 75 cm2 T-flask (Falcon, Corning incorporated, Life Sciences, Durham, NC, USA). After 4 days the culture medium was renewed, and 3 days after, when confluent, the cells were subcultured. Flow cytometry analysis of expression of characteristic markers of multipotential mesenchimal cells, CD29, CD44, CD73, CD90 and CD105 was conducted. The results confirmed that the ADSC were positive for all these markers (see supplementary information).

Chondrogenic differentiation

Preliminary tests revealed that in our model of electrical stimulation, the RF current distribution within the Petri dish and in the plated cells is influenced by the culture type. Namely, cells forming multi-cellular, spheroidal structures or micromasses were found to be less sensitive to the electrical treatment than those adopting a monolayer distribution on the dish surface (data not shown). This would be attributable to the fact that monolayer configuration allows homogenous exposure of all cells in the culture to the electrical stimulus, whereas when grouped into three-dimensional micromasses with relatively high electrical resistivity, the stimulus would reach only those cells located at the outermost layer of the spheroid. This methodological requirement, together with the fact that monolayer culture has been reported advantageous to chondrocyte differentiation within the first three weeks of incubation [33,34] led us to adopt monolayer culture as a suitable model for studying the early chondrogenic response to RF electrostimulation.

Discussion:

Previous studies by our group have shown that cyclic exposure to a subthermal dose of 448-kHz, sine wave electric current of the type applied in CRET therapy, can stimulate proliferation of ADSC cultures obtained from healthy human donors and grown in proliferating medium. These data suggested that the beneficial effects on tissue repair and regeneration attributed to CRET therapies could be mediated in part by electrically-induced stimulation of the proliferation of stem cells present in damaged tissues.

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