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Association of treadmill training and SHEDs transplant to treat experimental spinal cord injury

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Spinal cord injury is a disabling traumatic condition and available therapeutic approaches are poorly effective. Searching for new treatments, stem cell transplants and physical activity have been studied with a view to minimizing spinal cord injury in experimental rats. **Aim:** Assess the effectiveness of treadmill training and transplantation of human exfoliated deciduous teeth (SHEDs), isolated or in combination, evaluating the functional recovery, neuroprotection and neuroregeneration in spinal cord injury rats. **Methods:** Laminectomy was performed between T9 and T10 and a moderate contusion spinal cord injury was produced by the use of the NYU Impactor. Sixty male Wistar rats were distributed into 5 groups: sham, spinal cord injury, spinal cord injury treated with stem cells, spinal cord injury receiving treadmill training and spinal cord injury treated with treadmill and stem cells. Human dental pulp cells were implanted into the injury site 1 hour after injury, at a concentration of $0.3X10^6$ of cells diluted in 10µl of NaCl in 0.9%. Animals receiving exercise procedure were placed on a treadmill with a constant speed of 0.3 m/s beginning on the third day after spinal injury. The training was performed 5 days per week, 20 minutes per session for six weeks; functional assessment was performed using the Basso, Beattie and Bresnahan Scale (BBB). To evaluate the differentiation and cell survival on the spinal cord, glial scar density and neurofilament medium density, immunofluorescence was performed. **Results:** Transplantation of SHEDs, isolated and in combination with training promote functional recovery after spinal cord injury; however, treadmill training treatment alone was not effective. A decrease in the cystic cavity area was observed only in animals with combined therapy. Glial scar reduction and increased density of neurofilament medium (NF-M) were observed in the SHEDs group. The transplanted cells survived and were well integrated inside the tissue; however, there was no evidence of differentiation in the astrocytes and/or neurons. Increased cystic cavity areas as well as glial scar formation and low density of NF-M were seen in the treadmill training group. **Conclusion:** Transplantation of SHEDs promotes functional recovery after spinal cord injury, a reduction in glial scarring was possible and the neurofilament density was maintained. Treadmill training presented high density of GFAP+ and large cavity areas, with no functional recovery. The SHEDs associated with treadmill training were effective in improving functional recovery.

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Development of a model for the inhibition of recombinant adenovirus replication using shRNA anti-IVa2 and anti-hexon

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Background: A better understanding of the biology of Adenoviruses (Ad) could help shape the design and application of recombinant adenoviral vectors. Ad have a linear genome of double-stranded DNA, ranging in size between 30 and 40 kilobases. This genome is flanked on its left and right ends by ITRs; also present at the left end is the cis-acting signal for genome encapsidation mediated by a critical interaction with the IVa2 viral protein. The major viral capsid protein, hexon, is an important component of the virus structure, is expressed in the late phase of viral replication and is essential to form viral particles. We are developing a model for the inhibition of Ad replication by knockdown of IVa2 and hexon viral proteins by shRNA. The information gained in this model will help guide us in the design and future application of adenoviral vectors. **Aim**: Creation of a HEK293 cell line which expresses shRNA anti -IVa2 and anti-hexon to inhibit Ad replication. **Methods:** Lentiviruses expressing the shRNAs anti-IVa2 or anti-hexon were constructed by cloning oligos containing the shRNA sequences into Lego vectors. The Lego-dTomato-anti-IVa2 and Lego-eGFP-anti-hexon lentiviruses were produced and used to transduce the HEK293 and HEK293T cell lines and antibiotic resistant cells were selected. Homogeneous populations were established by cell sorting. **Results:** The lentiviral vectors encoding the shRNA sequences were successfully constructed and produced. Transduced HEK293 cells were positive for the reporter genes contained in the Lego-eGFP-anti-hexon and Lego-dTomato-anti-IVa2 vectors as confirmed by confocal microscopy. Analysis by flow cytometry showed that over 97% of HEK293anti-IVa2/anti-hexon cells were positive for dTomato and eGFP. After cell sorting, a homogeneous population was recovered where more than 99% of cells expressed both markers. Similarly, after sorting, the HEK293Tanti-hexon cell line was homogeneous with over 99% of cells positive for eGFP. **Discussion**: HEK293 and HEK293T cell lines containing the shRNA-bearing lentiviruses were successfully established. Our next challenge will be to assess the ability of the shRNA to mediate the knockdown of IVa2 and hexon expression and determine if adenovirus replication will be inhibited. Since adenoviral vectors replicate in HEK293 and HEK293T cell lines, halting this process by shRNA will be an especially rigorous test of our strategy. Support : Grant #2012/05066-7 São Paulo Research Foundation (FAPESP).

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Use of biomaterials with or without mesenchymal stem cell association for the treatment of burn animal models

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Short-and long-term complications in extensive deep dermal and full-thickness skin injuries may be severe and require adequate wound surface closure. The association of stem cells (SCs) with biomaterials promises to be the protagonist for future regenerative medicine in the treatment of tissue and organ lesions. Aiming at the utilization of the scaffolds of poly-D,L-lactic acid (PDLLA) associated or not with *Spirulina* biomass (PDLLA/*Sp*) (patent applied for) in skin wounds, mesenchymal SCs (MSCs) from kidney of mice were seeded onto nanofibers produced by electrospinning. The anti-inflammatory and antimicrobian effects of the microalga *Spirulina* are interesting for skin application. The biodegradable and biocompatible matrices produced were evaluated for morphology and fiber diameter by scanning electron microscopy. The molds for growing MSCs were implanted in mice with skin defects that mimic burns for up to 7 days. Five groups were tested: (1) PDLLA with MSCs; (2) PDLLA/*Sp* with MSCs; (3) PDLLA without MSCs; (4) PDLLA/*Sp* without MSCs and (5) animals injured without scaffolds (control group). For groups 1- 4, a protective

cover of 100 nm thick PDLLA scaffolds was used. The following healing characteristics were evaluated: fibroblast proliferation, the presence of mononuclear and polymorphonuclear cells, occurrence of fibrosis, extent of ulceration, re-epithelialization, presence of hair follicles and keratinization. The fiber diameter of the scaffolds obtained for PDLLA and PDLLA/Sp were 276±65.9 and 263±82 nm, respectively. Concerning the animal experiments, the scaffolds implanted in the animals tolerated the mechanical stress up to two weeks without breaking. In group 5, the wounds seemed to be more disorganized and with a larger bloody area when compared to the other groups. The histopathological parameters showed no difference between the groups in the time analyzed. After 7 days of the induction of the lesions, a similar presence of ulceration, inflammation and fibrosis among all the treatments was observed. In group 5, the wound surface was covered with fibrous tissue residues and below this layer, a large leukocyte infiltration and fibroblast proliferation was observed. In groups 1-4, inflammatory infiltration and, consequently, fibroblast proliferation was present under the cover of the nanofiber scaffold, and there was also a deposit of the remaining fibrous tissue. During this recovery time, no group showed signs of re-epithelialization, keratinization or presence of hair follicles on the lesion site. The scaffolds used in groups 1 and 2 were more densely populated by cells than groups 3 and 4 but it is also possible to note a cell population in these groups, most probably from the host animals. The scaffolds developed in this study demonstrated fibrous and porous structure similar to the natural extracellular matrix of the cells. Although there was no microscopical difference among all the groups, it is possible that more prolonged analysis would produce different results. Moreover, the microscopical analysis of groups 1-4 showed better cicatrization than in group 5. Moreover, the scaffolds serve as a bridge in groups 3 and 4. Therefore, from these results it is possible to suggest that these biomaterials promise to be suitable for use in TE. Financial Support: CNPq, FAPERGS and Stem Cell Research Institute.

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Genetic barcode sequencing as a simple tool for screening altered population dynamics of hematopoietic stem cells transduced with lentivirus

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Gene therapy has been successfully applied for the treatment of monogenic hematopoietic disorders, such as SCIDs, chronic granulomatous disease, β-thalassemia and Wiskott-Aldrich syndrome. However the insertional mutagenesis observed in some cases led to discussions about the security of the strategy used. For this reason, clonal analysis has proven to be very important for the assessment of vector safety and its impact on the patient's health. Unfortunately, there are few simple and quick methods that can reveal and quantify clonal expansion. Some currently used methods, such as LM-PCR and pyrosequencing, reveal population dynamics by detecting the integrated vector. While effective, these approaches require skills, equipment and resources that make them difficult to use in a less specialized laboratory. We have developed a genetic barcode containing variable sites that was used to label lentiviral vectors. This methodology provides a quantitative measure of clonality upon Sanger sequence analysis of genomic DNA recovered from transduced cells. The analysis is based on the evaluation of the area corresponding to the electropherogram peaks of each base at each variable site. Over time, alteration in the ratio of the peaks would indicate a change in population dynamics. In the polyclonal population, sequencing should reveal great variability, yet a decrease in population complexity would reduce barcode variability. That is, clonal expansion would be revealed when a single barcode (or a limited number of barcodes) is predominant in the population. Analysis of the barcode allowed quantitative measurement of the dynamics of transduced cells in culture tracked over time. That is to say, neither the vector nor the barcode altered cell growth, as expected. The IL2RG and LMO2 transgenes were inserted in the lentiviral vectors, barcode libraries generated and used to transduce mouse hematopoietic (c-kit$^+$) stem cells that were then transplanted in recipient mice. Peripheral blood was collected periodically and barcode sequencing revealed that either the empty or IL2RG encoding vector had no effect on the cell dynamics. In contrast, the LMO2 oncogene was associated with altered cell dynamics even though hematologic counts remained unchanged, suggesting that the barcode could reveal changes in cell populations not observed by the frontline clinical assay. Bone marrow immunophenotyping did corroborate the observation of changes in the cell population, indicating an accumulation of immature B-cells in the LMO2 group. In order to confirm these data, high resolution melting analysis (HRM) was performed. The HRM analysis also showed that the IL2RG or empty vector did not provide a selective advantage over time, since it gives exactly the same shaped melt curve for each condition. Instead, melt curves of the LMO2 population appear very different among each analyzed time point. Taken together, these data have proven that this new methodology can be applied for the follow up of clonal expansion *in vitro* and *in vivo* . We have presented a simple and sensitive method for the analysis of clonality, which could be easily used by any laboratory for the assessment of cellular behavior upon lentiviral transduction. Grants #2007/56495-7 and #2009/51386-0, São Paulo Research Foundation (FAPESP).

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Transduction of human melanoma cell lines with specialized adenoviral vector encoding human interferon -beta results in high levels of cell death

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The incidence of melanoma has continued to rise since 1990. Though melanoma only represents 4% of all skin cancers, its prognosis is especially grim once the metastatic disease state has been reached. However, the genotype of melanomas may provide an opportunity for therapeutic intervention. For example, 90% of cases retain wild-type p53 and a considerable number of these present deletions in the chromosome region that encodes interferon-β (IFN). In our previous work, we have developed adenoviral vectors where transgene expression is controlled by p53 and propose that such vectors would be especially interesting for the treatment of melanoma. We have used these vectors to transfer the mouse IFN cDNA in murine models, including melanoma, and have observed a significant ability of IFN to inhibit cell proliferation *in vitro* and *in vivo* (Merkel et al, 2013). In addition, we have shown that the application of IFN contributes to an anti-tumor immune response (unpublished data). Here we present our recent efforts to adapt this strategy to a model of human melanoma. To this end, the human IFN cDNA was cloned into an adenoviral vector that offers CAR-independent transduction due to the incorporation of the RGD tripeptide in the fiber as well as expression of the transgene under the control of p53. We expect that the AdRGDPGhIβ vector will provide expression of human IFN in response to cellular p53 activity. Reliable vector expression was confirmed by ELISA. Then SK-MEL-05 and SK-MEL-147 (human melanoma cell lines harboring wild-type p53) were transduced, incubated for 48, 72 or 96 hours of incubation and

then cell cycle alterations were assessed by PI/FACS analysis. We observed a time-dependent accumulation of hypodiploid cells in either cell line when treated with the AdRGDPGhIβ vector, yet non-treated or treatment with a GFP-expressing virus did not alter the cell cycle profile. At the final time point, more than 90% of cells treated with IFN were hypodiploid. This result suggests that endogenous p53 reliably induced vector expression and that IFN was an efficient inducer of cell death. We are especially encouraged by these results since our mouse models using Ad5 to transfer murine IFN were less efficient in inducing cell death than seen in the human model. At this time we are exploring the paracrine effects of this treatment, determining the mechanism of cell death as well as initiating a model of *in situ* gene therapy with human IFN. Grants #2010/15025-0 and #2011/50911-4, São Paulo Research Foundation (FAPESP).

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Evaluation of a new adenoviral vector expressing p53 in a feedback loop applied in a model of prostate cancer therapy

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In men, prostate cancer accounts for 28% of new cancer cases and is second in mortality due to cancer. Patients who develop only a localized tumor have a 5 year-survival rate of almost 100%, however, for patients with metastatic prostate cancer the rate is 31%, and mortality in 90% of cases is the result of systemic dissemination and metastasis. Viral vectors expressing p53 under control of a constitutive promoter (CMV) have been developed by other groups (AdCMVp53). Our laboratory has developed an adenoviral vector (AdPGp53) where expression of p53 is under the control of a promoter responsive to p53, establishing a positive feedback mechanism and should induce higher levels of p53 expression. Comparison of p53 expression mediated by AdCMVp53 and AdPGp53 in prostate cancer cell lines (DU145 and PC3) indicates that AdPGp53 induces higher levels of mRNA and therefore of protein expression as revealed by Real-Time-PCR, Western-blot and Immunofluorescence analyses. *In vitro* , AdPGp53 is also capable of reducing proliferation of prostate cancer cell lines and provoking accumulation of a hypodiploid population and induction of apoptosis as revealed by cell cycle analysis and Annexin V and propidium iodide staining. *In vivo* , subcutaneous inoculation of PC3 cells in athymic nude mice followed by *in situ* gene therapy with AdPGp53 resulted in reduced tumor growth and increased survival as compared to the AdCMVp53 cohort. A second improvement to the adenoviral vector was the modification of the fiber protein with the insertion of the RGD tripeptide, permitting the transduction of target cells even in the absence of the adenoviral receptor (CAR), generating a modified adenoviral vector where expression of p53 is under control of the p53-responsive promoter (AdRGDPGp53). This vector is currently being used to evaluate whether the union of gene therapy and chemotherapy applied to prostate cancer cell lines promotes a synergistic response in the induction of programmed cell death and reduction of tumor progression in an animal model. Grant #2011/21256-8 São Paulo Research Foundation (FAPESP).

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Construction and validation of an activating and inhibitory chimeric antigen receptor (CAR) system as a strategy to kill specifically tumor cells

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Background: The use of adoptive transfer of T cells for the treatment of cancer has been hampered by the low persistence and avidity of the infused cells and the difficulty to isolate and expand tumor-specific lymphocytes. The use of chimeric antigen receptors (CARs) allows the circumvention of some of these problems. CARs consist of an antigen recognizing unit (Fab fragment in form of scFv), a transmembrane region and an intracellular activating signaling domain. CARs redirect the specificity of lymphocytes, recognizing the target antigen with high affinity and in a MHC-independent fashion. Nonetheless, off-target responses owing to the recognition of the target antigen in healthy cells limit the broad application of this therapy. The membrane antigen CD19 represents a good target for the elimination of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) and anti-CD19 CAR transduced lymphocytes could be used clinically. However, the expression of CD19 as a pan B-lineage marker could lead to unwanted side effects, such as mature B cell depletion. **Aim:** Since mature B cells express both CD19 and CD20 antigens, we propose the creation of an inhibitory CAR directed to CD20, allowing the lymphocytes to discriminate between BCP-ALL and mature B cells. **Methods:** As a reporter system to test our hypothesis, Jurkat cells expressing the plasmid pGL4.30 – which express the luciferase protein controlled by a NFAT-responsive promoter – were generated. As target cells we used the cell line K562 modified to express CD19 (K5-19), CD20 (K5-20) or CD19 and CD20 (K5-19/20). Also the expression of the activation marker CD69 was evaluated by flow cytometry. The *Sleeping Beauty* transposon system was used to modify primary human T lymphocytes. The effector activity of these cells was evaluated by cytotoxicity and IFNg production assays. **Results:** Three different inhibitory αCD20-CARs containing signaling domains of the inhibitory receptors CTLA-4, PD-1 or BTLA were generated by SOE-PCR. In co-culture experiments, Jurkat cells expressing the αCD19- activating CAR 19BBz showed high activity of luciferase when cultured with K5-19 or K5-19/20. However, Jurkat cells expressing both the activating and inhibitory receptors showed a potent inhibition of luciferase activity when incubated with K5-19/20, while maintaining a high activity when cultured with K5-19 cells. Moreover, the three inhibitory CARs were able to inhibit the *de novo* expression of the activation marker CD69 induced by 19BBz in Jurkat cells. In primary T cells, the CAR 20PD1 was not able to inhibit the production of IFNg or the cytotoxic activity induced by 19BBz. Surprisingly, T cells expressing both 19BBz and 20PD1 showed preferential lysis of CD19+ CD20+ targets, suggesting that addition of PD1 signaling domain might augment T cell function in particular contexts. **Conclusion:** The results in Jurkat cells suggest that αCD20-CARs can inhibit the activation of lymphocytes mediated by αCD19-CAR. However, additional are required to evaluate the inhibitory activity of 20PD1 in primary human T cells. Experiments with CARs 20CTLA4 and 20BTLA are in progress and might allow the validation of the dual CAR hypothesis *in vitro* and *in vivo*. Financial Support: CNPq, FAPERJ, INCA-MS.

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Molecular mechanisms enrolled during endothelial-mesenchymal transition are dependent on the source of endothelial cells

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The epithelial-mesenchymal transition (EMT) is a biological process which allows polarized epithelial cells undergo multiple biochemical changes leading them to take on a mesenchymal phenotype. Endothelial-mesenchymal transition (EndMT) is an important and specialized form of EMT. In this process, there is a reduction in the expression of endothelial markers and increased expression of

mesenchymal markers. EMT/EndMT is involved in embryonic development and in some pathology such as fibrosis and cancer.Transforming growth factor-β (TGF-β) family plays pivotal role in EMT and EndMT process. However, it is unclear whether the anatomic origin of endothelial cells (ECs) reflects in differences on the cellular and molecular machinery activated during EndMT induction. Therefore, we investigate the activation of EndMT by TGF-β2 in ECs from distinct sources. We used two non-cardiac ECs (HUVECs, from umbilical vein; PAECs from pulmonary artery), and two cardiac ECs (CAECs, from coronary artery; AECs from aorta artery). Mammary epithelial cells (MCF-10A) were used as a model for EMT induction allowing the comparison between EndMT and EMT processes. After TGF-β2 treatment, the levels of p-SMAD3 were upregulated in MCF10A and non-cardiac ECs, demonstrating the activation of the TGF-β pathway. MCF10A cells underwent morphological and molecular changes consistent with EMT: the epithelial genes E-cadherin and EpCAM were transcriptionally repressed, and we observed an upregulation of the mesenchymal markers collagen type 1A1 (44x), MMP-9 (18x), ACTC1 (11x), SM22 (7x), fibronectin (5.5x) and N-cadherin (2x), as well as an increased expresion of the transcription factors Snail1 (14x) and Slug (2.7x). These transcriptional changes were also confirmed at the protein level. Interestingly, despite the activation of the TGF-β pathway, the non-cardiac ECs did not acquire a fibroblastic morphology and maintained the transcriptional levels of the endothelial markers PECAM-1 and VE-cadherin. The expression of mesenchymal markers and EMT-related transcriptional factors was only slightly upregulated in these cells: collagen type 1A1 (1.4x PAECs/1.6x HUVECs), SM22 (2x PAECs/2.7x HUVECs), ACTC1 (1.4x PAECs/27x HUVECs), fibronectin (1.6x in HUVECs), Snail (2x PAECs/HUVECs) and Slug (1.3x PAECs/3x HUVECs). Thus, despite some changes on the expression of mesenchymal markers, non-cardiac ECs did not acquire a full mesenchymal phenotype upon activation of the TGF-β signaling pathway. On the other hand, cardiac ECs underwent a clear transition from the endothelial to mesenchymal phenotype. In response to TGF-β2 treatment, these cells loss the endothelial marker PECAM-1 and markedly upregulated the expression of mesenchymal markers ACTC1 (838x AECs/7x CAECs), collagen type 1A1 (27x AECs/10x CAECs), SM22 (11x AECs/3.7x CAECs) and the transcription factors Snail1 (5.8x AECs/2.9x CAECs) and Slug (5x AECs). In conclusion, the anatomic source of ECs determines the activation EndMT upon TGF-β2 treatment. Therefore, we suggest that many of EMT regulators act in EndMT, but there may be specific and differential mechanisms enrolled during EndMT activation that are dependent on the anatomic origin of endothelial cells.

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Attachment capacity of adipocyte tissue mesenchymal stem cells in suture filaments: a new tool for the treatment of enterocutaneous fistulas

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Background and Aim: Enterocutaneous fistulas (EF) are difficult to resolve, surgical failure is frequent. Cell therapy could be a new approach. Mesenchymal stem cells (MSCs) are able to self renew, has high proliferative and immunomodulatory capacity and differentiate into several lineages. Adipocyte tissue (AT) is an easy MSCs source, lipoaspiration is a common procedure. Enterocutaneous fistula (EF) treatment with AT-MSCs was yet performed but sometime, fistula did not close completely. The aim of this study is to analyze if MSCs could attached in the suture filament to be used for EF treatment. **Methods:** AT was obtained from lipoaspirate procedures and was submitted to collagenase digestion. Cells were cultured in DMEM low glucose medium, with FBS during 3 days. At 80% of confluence, adherent cells were treated with trypsin and harvested with the medium above. At the 4ªpassages, cells were characterized by flow cytometry, confocal microscopy, differentiated to mesodermal lineages to confirm MSCs differentiation, submitted to karyotype and telomerase enzyme activity analyses to discharged tumor capacity. Experiments were performed with polyester suture filament. MSCs, 10^6cells, were fixed in the filament by adding fibrin glue (20uL Fibrinogen, 30uL Thrombin and 10uL Calcium Chloride). Filaments were led in the medium during 3 days. Samples were analyzed by confocal and scanning electron microscopy. Animal experiments were performed on 10 weeks old male Wistar rats divided into 3 groups: Control Group (CG) included 5 animals undergoing fistula formation alone. Injection Group (IG) consisted of 8 animals receiving 10^6 AT-MSCs injected around the suture line. Suture Filament Group (SG) consisted of 9 animals in which suture were performed using 4-0 Vicryl with 10^6 mesenchymal stem cells attached in the filament with fibrin glue. Fistula procedure were performed accessing the through a standard 7mm stab incision on the lower left side of the abdomen. Upon exposure, a 5mm enterotomy was performed and sutured to the abdominal wall in order to produce the fistula. To ensure normal closure of the fistula the opening in the cecum wall was fixed to the internal surface of the skin, without maturation, using four separate 4-0 Vicryl stitches (Poly J-304 Polyglactin 910 Ethicon). The fistulas were photographed on the day of operation and on the 3°, 6°, 9°, 12°, 15°, 17°, 19°and 21°day, in which they were anesthetized and sacrificed. Measure of the size of the fistula was performed using ImageJ software. Statistic comparison between the groups was performed by ANOVA. **Results:** Confocal and scanning electronic microscopy results demonstrated that the cells were able to attach to the suture filaments. Animal experiments showed that the average size reduction of the fistula area at the 21th day was 46.54% in the CG, 71.80% in the IG and 90.34% in the SG ($p<0,05$), demonstrating that MSCs were effective in promoting healing of an enterocutaneous fistula both as an injection or attached to the suture material. **Conclusion:** MSCs were able to attach to the suture filaments. Enterocutaneous fistulas sutured with filaments containing MSCs showed better recovery and healing. AT-MSCs adhered to suture filament might be a new and effective approach for enterocutaneus fistulas treatment.

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Identificação de um compartimento celular rico em células estromais progenitoras no tecido adiposo

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O tecido adiposo (TA) é considerado uma fonte promissora de células estromais mesenquimais (CEMs) para terapia celular, principalmente devido à facilidade de coleta e ao número elevado de células obtidas a partir da fração estromal vascular (FEV). Entretanto, a maior parte do conhecimento sobre a biologia das CEMs-TA advém de culturas celulares estabelecidas a partir da população aderente presente na FEV. A grande heterogeneidade destas culturas e as alterações na expressão de marcadores causadas pelo cultivo dificultam a identificação e purificação de células tronco/progenitoras. Recentemente, observou-se que a expressão do receptor de crescimento neural de baixa afinidade (L-NGFR) ou CD271 identifica uma população de células progenitoras mesenquimais na medula óssea humana *in situ* . Embora a FEV contenha células que expressam CD271, não se sabe se este marcador é também capaz de identificar células progenitoras no TA. Neste estudo, duas populações de células estromais foram isoladas por meio de *sorting* celular a partir da FEV não cultivada*:* $CD271^{pos}$ ($CD271^{pos}CD34^{pos}CD31^{neg}CD45^{neg}$) e $CD271^{neg}$($CD271^{neg}CD34^{pos}CD31^{neg}CD45^{neg}$). Ambas as populações

foram comparadas com a FEV total quanto à morfologia, potencial clonogênico e capacidade de diferenciação em adipócitos *in vitro* . Após a imunoseleção e cultivo, as populações celulares apresentaram morfologia fibroblástica e expressaram marcadores de superfície celular característicos de CEMs (CD90, CD73 e CD105). No entanto, o potencial clonogênico das populações celulares, avaliado através da quantificação de unidades formadoras de colônias fibroblásticas (CFU-Fs), revelou que a população $CD271^{neg}$ possui maior número de CFU-Fs em relação à população $CD271^{pos}$ e à FEV total (1,17%±0,07%, 0,45%±0,28%, 0,25%±0,07%, respectivamente). Em concordância com estes resultados, análises da diferenciação adipogênica *in vitro* através do cálculo da área diferenciada pelo *software* ImageJ e da recuperação do corante Oil red com isopropanol, revelou que a população $CD271^{neg}$ apresentou uma área maior de diferenciação em relação às células $CD271^{pos}$ e à FEV (10,50%±3,67%; 4,79%±2,23%; 2,86%±1,89%, respectivamente) e maior densidade óptica do corante oil red recuperado (0,261±0,007; 0,201±0,012; 0,135±0,002, respectivamente). Analisando a caracterização imunofenotípica da FEV total recém-isolada, observou-se que as células $CD271^{neg}$ contêm uma subpopulação celular $CD146^{pos}$, cuja expressão também é característica de células tronco mesenquimais na medula óssea. Notavelmente, as células $CD271^{pos}$ não expressam o marcador CD146. Este achado implica na possibilidade de que células $CD146^{pos}$ sejam as responsáveis por conferir o potencial clonogênico e de diferenciação observado na população $CD271^{neg}$. Portanto, nossos dados sugerem que a população celular $CD271^{neg}CD34^{pos}CD31^{neg}CD45^{neg}$, que contém uma subpopulação $CD146^{pos}$, possui maior capacidade clonogênica e de diferenciação em relação à população $CD271^{pos}CD34^{pos}CD31^{neg}CD45^{neg}$ e à FEV. Estes achados são relevantes para o estudo e aplicação terapêutica das CEMs, pois fornecem evidências capazes de auxiliar a identificação definitiva de células progenitoras mesenquimais no tecido adiposo.

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Assessment of tumor vasculature in a murine melanoma model after treatment with adenoviral vectors carrying interferon-beta and p19ARF

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The vasculature plays a central role in tumor progression and it represents a therapeutic target of great interest. Inhibition of angiogenesis has the potential to slow tumor progression and inhibit metastasis. To this end, anti-angiogenic therapies have been developed and have been shown to retard tumor growth. The main mechanism of action of these therapies is through modulation of VEGF (vascular endothelial growth factor), FGF (fibroblast growth factor) and its receptors. The Laboratory of Viral Vectors at the Cancer Institute of São Paulo State (ICESP) develops work in cancer gene therapy using the expression of interferon-beta (INFβ) and p19ARF mediated by recombinant adenoviral vectors. According to the literature, IFNβ changes the tumor vasculature through inhibition of angiogenic factors and p19ARF protein regulates angiogenesis via inhibition of HIF-1α. The influence of the combination of these vectors in angiogenesis has not been investigated in our experimental model. Thus, with this project we will evaluate the impact of INFβ and p19ARF combined treatment in modulating tumor angiogenesis of melanoma. We found that adenovirus expressing recombinant proteins of interest show high levels of transduction in murine endothelial cells (T-END) and murine melanoma (B16) as verified by immunofluorescence and ELISA. Our group also demonstrated *in vitro* that the combination of interferon-beta and p19ARF induces cell death and decreased tumor progression in murine models of melanoma. The *in vitro* evaluation of proliferation, tube formation and expression of angiogenic markers in endothelial cells will be performed after transduction with these viral vectors or upon co-culture with transduced B16 cells. For *in vivo* testing, we will perform *in situ* gene therapy experiments in a murine model of melanoma where tumorswill be collected and evaluated histologically to reveal changes in angiogenesis. With this project, we aim to reveal the impact of our gene therapy approach on tumor angiogenesis.

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Combined p19ARF and interferon-beta gene transfer to mouse melanoma cells is associated with bystander activity and a cooperative impact on gene expression

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Background: Loss of p53 function by genetic mutation or alterations in its pathway, such as p19Arf loss and/or mdm2 over-expression, inhibits cell death and is critical for tumor progression. Interferon-β (IFN), an immune-stimulatory cytokine, is also frequently lost in some tumor types, fact that could inhibit an anti-tumor immune response. Because of this, we propose that p19Arf and IFN gene transfer, especially in wild-type p53 tumor cells, could combat the primary tumor and metastasis. **Methods**: Recombinant adenoviral vectors with a p53-responsive promoter (PG) were constructed containing genes of p19Arf, IFN or combination of both. Their antiproliferative effect was evaluated in B16F10 cells in vitro (B16, mouse melanoma, p53 wt) by annexin/PI staining and MTT assays. Bystander effect was revealed by cell cycle analysis of populations transduced with different proportions of the viruses. RNA expression profile was evaluated by microarray analysis of treated samples. **Results and Discussion:** Cell death resulted from the combined transfer of the p19Arf and IFNβ genes(74% hypodiploid), yet single gene transfer yielded only half the number of dead cells. A similar result was seen by measurement of cell viability with MTT. Evidence for a bystander effect was revealed when approximately 50% hypodiploid cells were observed, even though only 10% of cells had been transduced with IFN. In a population of cells transduced with p19Arf, when 10% of them also expressed IFN, the number of dead cells increased to 68%, compared to transduction of p19Arf alone, which results in 45% hypodiploid cells. This indicates that p19Arf can sensitize cells to the IFN-mediated bystander effect. Microarray analysis revealed that combined, but not individual, transduction of B16 cells with the AdRGD-PG-p19Arf and AdRGD-PG-IFNβ vectors altered the expression of 1054 cellular genes. Among the genes upregulated by the combined treatment, their functions include: cell death and apoptosis, regulation of transcription, immune system activation, GTPase activity and DNA repair response. Furthermore, the combination of p19Arf and IFN downregulated genes related to: cell cycle, cell adhesion, chromatin organization, mitochondrial organization, cytoskeleton organization and melanin production. **Conclusion**: The use of p53-responsive vectors to express p19Arf and IFN represents a potential strategy for melanoma suppression. We have shown that complementation of the p53/Arf and interferon pathways may generate a strong bystander effect as well as synergistic gene regulation and that this could come to be a promising approach for cancer treatment. The combination of p19Arf and IFN is able to regulate many essential functions of the cancer cells, destabilizing cell cycle and cell organization in general, while promoting expression of genes related to cell death and factors that render cancer cells susceptible the immune system. Grants 2011/10656-5 and 2011/50911-4 Sao Paulo Research Foundation (FAPESP).

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Treatment with the combination of p19ARF and interferon-beta elicts a strong and sustained anti-tumor immune response

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In a recent work we have shown that the combined, but not the individual, application of Adenovirus vectors encoding the p19Arf (a tumor suppressor protein and the p53 functional partner) and Interferon-beta (IFNβ – a pleiotropic immune stimulatory cytokine) results in massive cell death of B16F10 cells (mouse melanoma) both *in vitro* and *in vivo*. However, the immune stimulatory capability of this combination was not investigated. Here, we explore a B16F10 tumor vaccine model to reveal that the anti-tumor immune response unleashed by this combination protects mice against tumor challenge and has the involvement of Natural Killers (NK) cells. We also show that this immune response can be maintained for a long period after vaccination and is more dependent on the functions of IFNβ than p19Arf. First, enhanced killing of B16 cell was confirmed by cell cycle analysis and annexin/PI staining. Next, we investigated if this combination could induce an anti-tumor immune response in a prophylactic mouse vaccine protocol, in which cells dying due to the p19Arf and IFNβ treatment were used as a vaccine agent against a tumor challenge with naïve B16 cells (applied seven days after the last vaccination). Tumor progression was markedly delayed as compared to control groups (PBS, live naïve B16 and dead B16). This protective effect was also observed when challenge was done 73 days later after the last vaccination, suggesting a protective immunological memory. To explore the use of these dying cells as a vaccine agent, different amounts of cells and frequencies of injection were tested in order to minimize tumor formation at the vaccination site. Tumor formation was abrogated only by the p19Arf and IFNβ combination and when applied in a single dose. The involvement of NK cells was revealed by injecting these dying cells in nude mice (high activity of NK cells), nod-scid mice (no activity of NK cells), immune competent C57BL6 (regular activity) and in dexamethasone (an immune suppressor of NK cells) immune suppressed C57BL6 mice. Tumor formation was completely abrogated in the immune competent C57BL6 and in nude mice. However, in the dexamethasone and nod-scid models, this response was lost, demonstrating the involvement of NK cells. Next, in order to determine if the protective effect was more dependent on IFNβ or p19Arf, mice were first vaccinated with cells treated with p19Arf, IFNβ or the combination and then challenged. In the p19Arf group, tumor progression was partially delayed as compared to control groups (PBS or dead B16 cells). However in the IFNβ and combination groups, tumor progression was significantly reduced. Nevertheless, a significant increase in survival was only observed in the combination group. Together, these findings evidence the capability of the p19Arf and IFNβ combination to elicit a strong and sustained anti-tumor immune response, possibly, by activating natural killer cells and an adaptive immune response mediated by CD4+ and CD8 T lymphocytes. Support: grant 2010/03958-2, São Paulo Research Foundation (FAPESP).

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Mensenchymal stem cells and their use in the reconstruction of thyroid cartilage using nanofibers as scaffolds

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Cancer of the larynx is very prevalent today and, in many cases, the only alternative is the entire removal of the organ. The aim of this study has been to establish a regeneration alternative of the thyroid cartilage in pigs, reconstructing the organ though the use of human mesenchymal stem cells (MSCs) from deciduous teeth pulp grown on nanofiber matrices. The scaffolds were produced by the electrospinning technique using PDLLA with fiber diameter and pore size of 276±65.9 nm and 2,569±1,279, respectively. Four one month old pigs were submitted to cervicotomy with exposure of the thyroid cartilage. A resection of 4.0 $cm^2$of cartilage was carried out in the right upper region of the cartilage where, in this region where there was an absence of cartilaginous tissue, the scaffold with MSCs (SCA+MSCs) was sewn. The same type of lesion was induced in the upper and lower left regions of the cartilage in the same animal. In the left upper region, a scaffold without MSCs (SCA) was surgically implanted. In the left lower region, no biomaterial was implanted and the defect was left open (control- C). Thirty days after the implant of the biomaterials, the animals were put down and macroscopic and microscopic analyses were carried out with the aim of characterizing the three lesion sites. The average extension of the cartilaginous neo-formation of group C was 136.3μm (±9.6) and 387.7μm (±43.2) in the SCA group, presenting a significant statistical difference ($p<0.01$). The analysis carried out on the lesion site sections of the cartilage of the larynx of the animals from group SCA+CT showed an average of the extension of neo-cartilage of 825.4μm (±122.1), showing a more extensive area of neo-cartilage when compared to the other groups. These results demonstrated a high significantly statistical difference ($p<0.001$) when compared with the C and SCA groups. Cartilage is known as a tissue without any regeneration capacity because, after the removal of cartilage, only fibrosis occurs. Regeneration of part of the tissue, as happened in the present study, is considered a successful result. In 100% of the cases where SCA+MSCs were used, a significant success in the cartilage growth and closing of the lesion in the thyroid cartilage was obtained, when compared to the other two groups where MSCs were not used. The presence of the MSCs on the scaffolds for cartilage reconstruction was very important in the correction of the surgical defect, demonstrating that this approach is a promising tool for tissue regeneration in cases of laryngectomy.

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Use of gene transfer for cancer immunotherapy combining p19-induced cell death with interferon immunomodulatory effects

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It is now fully accepted that the success of some traditional chemotherapies not only rely on cancer cell death, but also on the induction of an immune response. Based on this knowledge, we are developing a gene therapy using adenoviruses coding for p19ARF as inducer of cell death, and interferon β (IFN β) as an immunostimulatory molecule. Cell death induction by p19ARF usually occurs through interaction with the MDM2 protein, inducing stabilization and accumulation of p53, which then assumes its function. There are several roles of IFN β in the immune system, and it is known that IFN β also has cytostatic and pro-apoptotic effects. *In vitro* transduction of Lewis Lung Carcinoma (LLC) cells shows that the introduction of p19 alone is responsible for the induction of cell death, and its association with IFN β does not provide a synergistic effect. However, when LLC tumors are implanted in C57BL/6 mice and treated with intratumoral injections of adenovirus coding for GFP, IFN β, p19ARF or IFN β + p19ARF, the effect of the therapy shows that IFN β has an key role in reducing tumor growth, and its association with p19ARF does not have synergistic or additive effects (GFP: 3997 mm^3, IFN β: 1825 mm^3, p19ARF: 2790 mm^3 e IFN β +p19ARF: 1795 mm^3). The abrogation of the antitumor effect of IFN β in nude mice confirms that the antitumor response to IFN β relies mainly on immunomodulatory properties and not on cytostatic effects. As mentioned above, the association p19ARF + IFN β gene transfer failed to show a beneficial effect on growth of primary tumor compared to single gene transfer in immune competent C57BL/6 mice. However, this association of genes was responsible for the induction of a potent immune response, as demonstrated by the reduction of tumor growth when rechallenge of treated mice was performed (IFN β:315 mm^3, IFN β+p19ARF: 123mm^3). Furthermore, the association of p19ARF and IFN β could reduce the appearance and size of the tumors in vaccination protocols (IFN β: 562 mm^3, p19ARF: 754 mm^3 e IFN β +p19ARF: 295 mm^3). Preliminary studies suggest that the improved immune response does not involve an increase in the recruitment of antigen presenting cells in the tumor site. In conclusion, we have shown that the combined treatment of p19Arf and IFN β gene transfer provides an advantage with respect to immune modulation.

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Sensor of caspase 3-dependent apoptosis and its application in the detection of adenovirus AdCDKN2AIRESp53 mediated cell death

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Although lung cancer is one the most common tumor types where p53 gene therapy has been applied and promising results have been achieved, improvements to this approach are necessary. In these tumors, functional loss of p53 and CDKN2A (p16) genes is a common feature and may prove to be a significant barrier to the antineoplastic apoptotic cell response. Deregulated cell death, including the apoptotic pathways, is a common feature in cancer and modulation of this cellular response has proved to be an effective therapeutic strategy. For example, many cytotoxic agents, including p53, are potent anticancer therapeutics thanks to their strong pro-apoptotic action. At the late stage of cancer progression, p53 is hijacked in all forms of tumors, yet active p53 is essential for effective chemo- or radio-therapy. When p53 function is turned on, one of the more drastic cell responses is the induction of caspase 3 dependent programmed cell death. Loss of CDKN2A is debilitating since, among other functions, it undermines Rb-mediated cell cycle control. Our previous results showed that simultaneous re-introduction of p53 and CDKN2A mediated by a bicistronic adenovirus, AdCDKN2AIRESp53, into H1299 non-small cell lung cancer cells *in vitro* and in a xenografic model revealed a dramatic induction of cell death that was superior to single p53 or CDKN2a gene transfer. For this reason, we are interested in real time monitoring of caspase 3 activity as a reporter of pro-apoptotic efficacy of AdCDKN2AIRESp53. In this study, we have used the lentivirus LvGFP-DEVD-ssGluc, developed by the Thannos laboratory, to sensorize H1299 (p53 null) cells. Upon apoptosis, the DEVD peptide is cleaved in response to caspase 3 activation, freeing ssGluc that can be detected in the culture medium over time. Our assays revealed that simultaneous transfer of the p53 plus CDKN2A tumor suppressor genes resulted in caspase 3-mediated apoptosis at levels that were much higher as compared to treatment with either of the single tumor suppressor genes. With this promising result, we plan to establish an in vivo model of in situ gene therapy where cell death induced by our treatment approach may be monitored in live mice. In addition to the H1299 experimental model described here, we hope to sensorize a large number of cell lines and use the ssGluc strategy for high throughput screening of the therapeutic potential of AdCDKN2AIRESp53. São Paulo Research Foundation (FAPESP).

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Religiosidade e células-tronco: uma investigação das representações sociais (morais) de indivíduos religiosos acerca das terapias celulares

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Introdução: A notoriedade das pesquisas com células-tronco (CT) relaciona-se a suas potenciais contribuições para questões de saúde e qualidade de vida, embasada num regime de expectativas de tratamento de doenças até o momento incuráveis. Pesquisa com CT tem repercussões em questões ético-morais, políticas e sociais. Alguns estudiosos consideram os "atores religiosos"centrais na rede de influências sobre essas questões, pautando uma ética compatível com sua doutrina. Frente ao conflito entre ciência vs. religião, os autores se propuseram a investigar e analisar valores morais dos indivíduos religiosos acerca das pesquisas com CT. **Métodos**: Trata-se de uma pesquisa transversal e descritiva. Tem como sujeitos indivíduos que responderam ao questionário disponibilizado pelos autores. O processo de amostragem foi por conveniência utilizando a técnica não probabilística *snowball sampling.* O questionário contendo 25 questões fechadas, foi disponibilizado online em Formulário no Google Docs®. Resultados obtidos foram em SPSS®versão 18. Este projeto foi avaliado e aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Ciências da Saúde da Universidade de Brasília. **Resultados:** Foram entrevistados 147 indivíduos (95 mulheres, 52 homens), entre 15-57 anos. Católicos tiveram mais representantes, seguidos por espíritas e evangélicos. 116 sujeitos se consideram religioso praticante e 84 já discutiram o tema pesquisa e tratamentos com CT em suas igrejas. A maioria dos católicos disse que sua religião não proíbe ou recrimina pesquisa (59,3%) e tratamento (50,4%) com CT adultas, mas sim com CT embrionárias. Apesar de parte considerável dos católicos não se mostrar completamente informado, esse grupo tem razoável conhecimento sobre os princípios defendidos por sua Instituição. 89,8% dos entrevistados concordam que os seus líderes religiosos

tem papel determinante ou relevante na formação de opinião de seus fiéis. A maioria dos entrevistados se sujeitaria a tratamento com CT extraídas dele mesmo ou de um doador; as opiniões foram mais divididas quando com CT embrionárias. 66,7% dos participantes consideram tratamentos com CT adultas ou embrionárias moderadamente seguros. Muitos respondentes doariam suas próprias CT para pesquisa e banco de células, mas não seus gametas. **Discussão e Conclusão**: A maior representatividade de católicos não constitui viés para o trabalho, cujo objetivo foi avaliar a opinião de religiosos. Católicos apresentaram-se bem informados, talvez pelo fato de muitos serem praticantes e/ou possuírem elevando grau de instrução, porém dados revelam uma ainda necessária alfabetização científica dos mesmos. Demonstrou-se a importância que comunidades religiosas têm sobre seus fiéis no que tange às terapias celulares. Cerca de 25% dos católicos responderam que participariam de terapias com CT embrionárias, o que vai de encontro aos dogmas da religião. Alguns participantes consideram as terapias celulares inseguras até o momento. Conclui-se que formação religiosa desempenha papel central nas decisões morais dos indivíduos, mas que estes ainda possuem certa autonomia para tomar decisões referentes à tratamentos de saúde, particularmente, na adoção de terapias celulares com CT.

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In vitro analysis of a new soluble trail variant in head and neck squamous cell carcinoma cell lines

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Head and Neck Squamous Cell Carcinomas (HNSCC) refer to malignant tumours originated from epidermoid cells, arising from the mucosal lining of the upper aerodigestive tract, salivary glands, paranasal sinuses, and skin of the head and neck. It represents the sixth world cause of cancer-related death. Despite vigorous molecular and clinical knowledge about HNSCCs, the median survival of newly diagnosed patients remains at 60% in 5 years. In this work, our aim was to analyze *in vitro* a new soluble trimeric form of the TNF-related apoptosis-inducing ligand recombinant gene (Apo2L/TRAIL) for HNSCC treatment. Trimerization of the Apo2L/TRAIL extracellular domain is forced with the Foldon domain of the bacteriophage T4 fibritin gene. FaDu, JHU28, HN13 and SCC25 HNSCC cell lines were transfected with a plasmid (pCI.Neo.sfTRAIL) containing the recombinant TRAIL gene, using Lipofectamine2000 reagent (Life Technologies). Empty pCI.Neo plasmid (Promega) was used as negative control. 72 hours after transfection, cells were evaluated for apoptosis and cytotoxicity using Apotox-Glo kit (Promega). Western-blot analysis was done for Caspase 8 and 9, PARP and Apo2L/TRAIL. Results were normalized by viability. *t* test was applied in all cases. Apoptosis was evidenced in HN13 (p value = 0.0411) and SCC25 (p value = 0,0022). No evidence of cytotoxicity was observed between sfTRAIL and negative control transfections. Data indicates that this new soluble trimeric form of the Apo2L/TRAIL death receptor ligand may become an interesting molecule for cancer gene therapy.

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Feasibility of gene transfer with non-viral vectors in murine models of sepsis

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Treatment of sepsis remains as one of the main challenges of biomedical science. In the last two decades, while the incidence of sepsis increased due to higher life expectancy and the development of more aggressive therapies for cancer, several target-specific therapies for sepsis failed to translate into real benefits in large phase 3 trials. Nonetheless, the increasing knowledge about the cellular and molecular mechanisms of sepsis and septic shock continue to reveal several new potential therapeutic targets that can be explored in the future. One of the challanges of previous target-specific treatments for sepsis was the short half-life of agents such as recombinant activated protein C, in the range of a few minutes. Gene transfer strategies can overcome this limitation, by providing a platform for longer expression of secreted therapeutic proteins. On the other hand, the transient nature of sepsis precludes the use of gene transfer strategies that lead to long-term expression such as viral vectors. In this context, the use of non-viral vectors emerges as an attractive strategy for the treatment of sepsis, provided that sufficient levels of expression of any therapeutic gene can be obtained. Here we evaluated the feasibility of gene transfer with non-viral vectors in the mice endotoxemia model. Two different constructs were used to evaluate relative levels of gene expression: a lacZ expression plasmid driven by the CMV promoter, and a coagulation factor IX (Padua (FIX-R338L) expression plasmid driven by the hAAT liver-specific promoter. C57Bl/6 mice were challenged with LPS and gene transfer was performed 6 hours thereafter, so as to mimic the time-point when sepsis treatments would be initiated. Fifty micrograms of plasmid were injected in the tail vein using the classical hydrodynamic transfection protocol, as well as a less aggressive protocol, that could in principle be translatable to the clinical setting. Gene expression was evaluated 72 hours after gene transfer by histochemical and coagulation activity (by a blinded investigator) assays respectively. Coagulation factor IX activity levels (FIX:C) were significantly lower in non-transfected LPS-challenged mice compared to non-transfected controls, suggesting that endotoxemia decreases FIX:C levels. The higher FIX:C levels (2-fold higher than controls) were observed in control mice submitted to hydrodynamic transfection. LPS-challenged mice presented 1.7-fold higher FIX:C levels than non-transfected LPS-challenged mice ($P<0.01$). Moreover, mice that were exposed to a less aggressive intravenous transfection protocol presented FIX:C levels that were 1.4 fold higher than their controls ($P=0.04$). Liver-expression of beta-galactosidase also demonstrated the feasibility of gene transfer in LPS-challenged mice, although gene expression was not blunted in LPS-challenged animals. Our results suggest that non-viral vectors are an attractive platform for the expression of secreted proteins in the context of sepsis. Additional experiments are underway to evaluate whether pathological caractheristics of sepsis such as the breakdown of the endothelial barrier can indeed facilitate liver transfection, as suggested by our results.

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Avaliação do entendimento (técnico e moral) de médicos e estudantes de medicina sobre células-tronco

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Introdução: Temas envolvendo inovações tecnológicas em saúde e sua aplicabilidade na vida das pessoas ganharam espaço na última década. As pesquisas com células-tronco (CT) talvez sejam um dos assuntos que mais ganharam projeção na mídia e geraram debate em âmbito nacional. Há diversos tipos de CT, com os mais variados potenciais de uso terapêutico, mas todas têm como objetivo primário reparar ou reconstruir tecidos ou órgãos danificados por doenças. Dentro da sociedade, o grupo dos médicos e demais profissionais de saúde, que irão, no fim da cadeia, ser responsáveis pela utilização dessa tecnologia, fazendo a aplicação desse conhecimento desenvolvido no laboratório, é um segmento de importante relevância e cujo conhecimento sobre a área ainda não tinha sido avaliado. Este trabalho tem como objetivo geral a investigação das representações

sociais (morais) do segmento de profissionais de saúde (médicos e estudantes de medicina) acerca das pesquisas clínicas com CT no Brasil. **Métodos**: A pesquisa foi realizada por meio de um questionário on-line, com o público alvo de estudantes de medicina/médicos do DF, divulgada por meio de redes sociais ou contato direto e disponibilizado para respostas entre os meses de março e maio de 2013. A análise estatística das respostas foi feita com o programa SPSS versão 18. Deve-se salientar que foi utilizada uma estatística descritiva simples, dada a homogeneidade da amostra. **Resultados:** Foram coletadas 56 respostas, 45 de estudantes de medicina e 11 de médicos. Sendo 57,1% do sexo masculino e 42,9% do sexo feminino. Não houve diferença significativa no padrão de respostas entre sexos ou entre médicos e estudantes de medicina. Os entrevistados conheciam todos os tipos de CT e afirmam que o mais utilizado em pesquisas eram CT do cordão umbilical (37%), e os melhores resultados ocorriam nos tratamento com CT embrionárias (37,5%). Doenças crônicas, danos traumáticos e problemas congênitos foram citados como condições que se beneficiariam com terapia com CT. Pouco mais de um terço dos entrevistados recomendaria hoje o tratamento com CT pra familiares ou pacientes. Boa parte não vê problemas éticos no uso de CT (60,7%). Mais de 75% doariam seus gametas ou suas CT para bancos de pesquisa. A maioria dos entrevistados respeitaria a opinião do paciente se este fosse contrário ao tratamento. Uma parcela de 10,7% afirmou que o atual estado de pesquisas com CT já deixa a terapia segura. **Discussão e Conclusão:** Os entrevistados demonstraram conhecer os tipos de CT, mas não tiveram opiniões concordantes com a literatura quanto ao tipo de célula mais usada na pesquisa nem ao tipo celular que apresentou melhores resultados. As condições clínicas que se beneficiariam foram concordantes com a literatura. No atual contexto de medicina baseada em evidências, os profissionais de saúde devem manter uma atualização constante para evitar que tenham seu juízo influenciado por pressões políticas ou financeiras. A informação é a melhor ferramenta que o médico pode ter. CT continuam sendo um tema polêmico e cabe ao médico, como formador de opinião e futuro usuário dessa possível forma terapêutica, estudar e se atualizar. Medidas de formação continuada em âmbito nacional podem ser tomadas, como a criação de disciplinas de Medicina Regenerativa nas faculdades.

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Perfil dos ensaios clínicos brasileiros com células-tronco

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Introdução: Na última década, houve crescente investimento em pesquisa na área da biomedicina regenerativa no Brasil, com significativo fomento por parte do governo brasileiro, acompanhado da aprovação da Lei nº11.105 no período em questão. Esse avanço se deve às possibilidades terapêuticas que este campo – que agrega pesquisa básica e clínica – oferece para o tratamento de mais de 80 diferentes condições clínicas, uma lista que pode ser estendida, segundo perspectivas otimistas de pesquisadores da área. O presente trabalho propõe-se a realizar uma investigação sobre os ensaios clínicos com células-tronco (CT) no país e visa caracterizar técnica dos modelos em terapias celulares; avaliar os resultados das terapias celulares com CT com base nos dados publicados; e identificar fragilidades nos desenhos experimentais de ensaios clínicos com CT no Brasil. **Métodos**: Estudo retrospectivo, documental e em bases de dados nacionais e internacionais. Para o presente estudo, foram realizadas pesquisas no banco de dados do *Clinical Trials* em busca de informações gerais sobre os ensaios clínicos com células-tronco no Brasil e no mundo. Foi realizada uma pesquisa abrangente com as seguintes palavras-chave: “stem cell”e “Brazil”. Dentre os 45 projetos brasileiros obtidos na pesquisa eletrônica, 22 estudos foram analisados após a aplicação dos critérios de inclusão/exclusão. **Resultados**: O perfil dos ensaios clínicos analisados neste estudo mostra uma centralização das pesquisas nos estados de São Paulo e Rio de Janeiro. Apenas 5 dos estudos eram randomizados e 7 encontravam-se concluídos. O foco dos ensaios clínicos se concentra nas áreas da saúde que representam os piores índices de morbimortalidade no país. Assim, as doenças cardiovasculares ocupam o primeiro lugar tanto no número de ensaios realizados (31,82%), como na proporção de pacientes e instituições envolvidos. Em seguida, destacam-se as afecções da retina (18,18%), do sistema nervoso central (13,64%) e das doenças pulmonares (9,09%). Apesar da Lei nº11.105, a maioria dos grupos de pesquisa continua trabalhando com células-tronco adultas. **Discussão e Conclusão**: As pesquisas com células-tronco no Brasil contaram com um ambiente favorável para o seu desenvolvimento, tendo como peça fundamental o grande investimento feito por parte do governo. O perfil dos ensaios clínicos analisados demonstra a necessidade da realização de maior número de ensaios clínicos randomizados, duplo-cegos, estatisticamente significantes, pois é a partir de seus resultados que a eficácia do tratamento de doenças com células-tronco poderá ser comprovada e incorporada como método terapêutico.

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Validação de procedimento de seleção positiva de células CD 34+ utilizando buffy coat de cordão umbilical

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Introdução: Transplante haploidêntico de células progenitoras hematopoéticas com seleção positiva de $CD34^+$ é uma opção terapêutica no tratamento de doenças benignas em que não se dispõe de doador HLA full match. O enriquecimento com células $CD34^+$ se processa mediante a separação magnética da fração $CD34^+$, marcada com um reagente monoclonal anti$CD34^+$, a partir de um produto de aférese convencional. O protocolo desenvolvido originalmente para a seleção é descrito apenas para CPH coletadas por aférese de doadores mobilizados com fator de crescimento de granulócitos. Neste trabalho, descrevemos um protocolo de validação de seleção imunomagnética de células $CD34^+$ utilizando buffy coat de cordão umbilical. **Métodos**: O instrumento utilizado para seleção foi a CliniMACS (Miltenyi Biotec). O produto a ser selecionado foi obtido a partir de duas unidades de sangue de cordão umbilical e placentário, ABO e Rh isogrupo, com TCN total de 2976×10^6, percentual de células $CD34^+$ de 0,391% e total de CD34 de $11,63 \times 10^6$, volume de 200ml. O produto foi deseritrocitado por centrifugação, lavado com solução de PBS e albumina a 0,5% para remoção do excedente de plaquetas, com centrifugação a 200G, à temperatura ambiente por 15 minutos, sem breque, conforme recomendado no protocolo original CliniMACS. A seguir, processou-se com a marcação da fração $CD34^+$, adicionando-se o reagente magnético anti $CD34^+$ ao produto, mantido em incubação em agitador orbital por 30 minutos. Processaram-se mais duas lavagens para remoção do excesso de reagente com solução de PBS e albumina a 0,5 %, a 200G, em temperatura ambiente, por 15 minutos. O sobrenadante foi removido de modo a deixar um produto final com volume de 150 ml. TCN total de 1989×10^6(recuperação de 67%) e $CD34^+$de $9,15 \times 10^6$ (recuperação de 78%). O produto foi então selecionado na coluna magnética utilizando-se o kit padrão, que permite a seleção de até 60×10^9, sem intercorrências. **Resultados:** Obtivemos um produto final com $9,87 \times 10^6$de células nucleadas, com $CD34^+$ de $8,56 \times 10^6$(86,77%), com recuperação em relação ao início de 74% e 93% em relação ao pós lavado. Realizamos também contagem de CD3+ no produto final, 0,18%, que corresponde a $3,7 \times 10^6$. Foi feito ensaio de unidades formadoras de colônias (CFU)

com plaqueamento nas concentrações de 0,1, 0,2, 0,5 e 1,0x10^4 de CD34$^+$. Todos os CFU cresceram com sucesso, sendo que a leitura no 14º dia mostrou crescimento tão expressivo, que impediu a contagem adequada das mesmas. **Conclusão**: A seleção positiva de CD34 se processa com sucesso utilizando-se como produto inicial células progenitoras de sangue de cordão umbilical e placentário, o que o torna uma fonte factível para realização de protocolos de validação de instrumentos de seleção positiva.

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Avaliação da ativação de linfócitos T por receptores quiméricos de antígeno (CARs) e mirnas

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O tratamento do câncer utiliza estratégias baseadas em agentes quimioterápicos, radioterapia e cirurgia. Estas estratégias, no entanto, apresentam limitações e uma fração dos tumores não responde, necessitando alternativas terapêuticas. A imunoterapia utiliza componentes do sistema imunológico para o tratamento de câncer, como anticorpos monoclonais, a transferência adotiva de linfócitos e, mais recentemente, a modificação genética de linfócitos para eliminação e obtenção de uma resposta específica contra as células tumorais. A leucemia aguda é uma neoplasia que acomete células B, população que expressa a molécula CD19 na sua superfície. Este antígeno vem sendo utilizado como alvo de linfócitos modificados geneticamente com Receptores Quimérios de Antígenos (CARs). Estes receptores reconhecem o CD19 a partir da porção Fab de anticorpo, que está fusionada a uma região transmembrana e uma porção intracelular de sinalização com os domínios de ativação da cadeia zeta do CD3 e da molécula 4-1BB, sendo portanto denominado 19BBζ. Boas respostas terapêuticas em tumores hematológicos têm sido descritas na literatura com esta abordagem, embora ainda haja espaço para melhoras no sistema. O microambiente tumoral é hostil aos linfócitos antitumorais, isso ocorre pela expressão de moléculas inibitórias, como PD-L1, mediadores químicos inibitórios. Visando aumentar a resposta anti-tumoral pretendemos explorar a associação do 19BBζ com microRNAs (miRNAs), pequenos RNAs não codificantes envolvidos na regulação da expressão gênica em diferentes tecidos e descritos recentemente também como moduladores da ativação de linfócitos T. Neste contexto o miR-181a é um candidato interessante para co-expressão com CARs, pois sua superexpressão reduz o limiar de ativação dos linfócitos T mediante a diminuição de diversas fosfatases da via do TCR, como DUSP5, DUSP6, SHP2 e PTPN22. O objetivo deste trabalho é expressar a molécula 19BBζ e superexpressar o miR-181a em linfócitos T e realizar ensaio funcional destas células. Para isso, células mononucleares do sangue periférico de doadores saudáveis foram modificadas geneticamente pelo sistema de transposon *Sleeping Beauty.* Neste sistema o gene de interesse é flanqueado por sequências ITR reconhecidas e clivadas pela enzima transposase, que posteriormente insere o transgene no genoma celular. Para expressar o receptor 19BBζ e superexpressar o miR-181a, construímos um sistema de expressão bidirecional. Posteriormente utilizamos citometria de fluxo e qPCR para avaliar a expressão do CAR e do miR-181a e seus alvos, respectivamente. Os linfócitos gerados foram caracterizados quanto ao perfil de população de memória, CD8+ e CD4+, além de avaliados quanto à expressão de marcadores de ativação à atividade de lise tumoral *in vitro* utilizando como alvo a linhagem pré-B Nalm-6.