Palavras-chave: Células-tronco; Metodologia; Cultivo; Imunofenotipagem; Citometria de fluxo; Diferenciação.

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ENGINEERED CD19-CAR NK CELLS AS AN OFF-THE-SHELF ALTERNATIVE TO B CELL LEUKEMIA AND LYMPHOMA TREATMENT

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Aims: Chimeric antigen receptor-modified T (CAR-T) cells have been successfully used worldwide for the treatment of hematological tumors. In 2019, our group successfully treated the first patient in Brazil. However, their wide application is limited by inherent risks such as graft-versus-host disease and the amount of time it takes to produce CAR-T cells. Allogeneic CAR-Natural Killer (NK) cells can be used as universal products and may be easily available off-the-shelf for clinical application. Considering the importance of CAR-NK for clinical use, the aim of this study is to develop novel therapy to harness the potential of NK cells against leukemia and lymphoma, and to further enhance their effector function by both redefining their specificity and enhancing their potency. For that, we developed a procedure for the transduction and *ex vivo* expansion of NK cells from three different sources: NK-92 lineage, peripheral blood (NK-PB) and cord blood (NK-CB). In addition, we evaluated if the cytotoxicity of NK cells can be augmented by the expression of a 4th-generation CD19-CAR developed in our laboratory. **Methods:** NK-cell resistance to transduction is a major technical hurdle for developing NK-cell immunotherapy. So, we tested two different backbones to improve the transduction rates and developed lentiviral vectors expressing anti-CD19 CAR and IL-15 or IL-27. The transducing efficiency was measured by flow cytometry using anti-F(ab')2 antibody. To assess NK cytotoxicity, we compared *in vitro* potential of CAR-NKs to kill Raji and NALM-6 CD19+ cancer cell lines at multiple E:T ratios by using two methods: Europium Solution assay and the Incucyte Live-Cell analysis assay. **Results and discussion:** NK cells were successfully and stably transduced with two lentiviral backbones. However, the backbone with the promoter SFFV presented better results, and it was used to built our CAR-constructions. The transduction efficiency was assessed 48h after transduction, and it was 28% for CAR.19-IL-15 and 39% for CAR.19-IL-27 in NK-92 cells and for NK-PB and NK-CB cells, the transduction efficiency was around 20% for CAR.19-IL-15. After 21 days in culture, the percentage of CAR-NK cells progressively decreased. Further, we evaluated the CAR-NK cells *in vitro* cytotoxicity potential. CAR-NKs had a higher killing potential against Raji and NALM-6 CD19+ cell lines than non-transduced NK cells. To better evaluate the expansion potential of transduced NK cells, CAR-NK-92 cells were enriched, by using magnetic beads, to 98% of NK cell positive for CAR expression. The sorted CAR-NK-92 cells were able to expand and maintained a stable CAR expression (96%–98% of CAR-NK) during 74 days. The enriched CAR-NK cells showed a higher killing potential against CD19+ cell lines, compared with non-transduced cells. **Conclusions:** We developed two new functional CAR vectors. In addition, we generated CAR-NKs from three different sources with their anti-tumor activity increased against CD19+ cells. Next, we intend to use cytokines to improve *ex vivo* CAR-NK cell expansion, and to test their *in vivo* therapeutic potential. These results are the foundation for the establishment of a platform to produce effective CAR-NK cells for cancer immunotherapy. **Support:** FAPESP 2019/25309-0, 2013/08135-2, 2008/578773, CNPq 573754-2008-0; Capes (88887.140966/2017-00 and 88881.199630/2018-01).

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ESTABELECIMENTO DE UM BIOPROCESSO PARA PROLIFERAÇÃO EM LARGA ESCALA DE CÉLULAS ESTROMAIS MESENQUIMAIS DERIVADAS DE TECIDO ADIPOSO HUMANO

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O emprego de células estromais mesenquimais (MSC) tem sido considerado uma alternativa terapêutica promissora em estudos pré-clínicos e clínicos. Neste contexto, o tecido adiposo destaca-se como uma importante fonte para isolamento e cultivo de MSC. Para emprego de MSC em triagens clínicas é necessário um grande número de células da ordem de $1{,}0\times10^8$ células/paciente. Para que se atinja tal concentração é necessária a proliferação celular *in vitro*, no entanto, o processo de proliferação implica na manutenção das células em um microambiente artificial que pode induzir efeitos genotóxicos e afetar a viabilidade celular, interferindo diretamente na segurança e eficácia da terapia celular. Em função destes aspectos, objetivou-se com este estudo o estabelecimento de um bioprocesso para proliferação de células estromais mesenquimais derivadas de tecido adiposo humano (hADSC) em biorreator tipo tanque agitado, visando estabelecer um sis-