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Cell Therapy 2019_ Human CAR-NK cells: A new non-viral method allowing high efficient transfection and target cell killing_ Ingegnere T _ IRCSS Bambino Gesu Pediatric Hospital_ Italy

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Cell-mediated immune responses play a central role in the control of infections and tumor growth. In particular, cytolytic T lymphocytes (CTL) and natural killer (NK) cells are fundamental effectors against virus-infected, tumor and leukemia cells. They patrol our bodies for abnormal cells like cancer and destroy them. But cancer cells can make themselves invisible, making it much more difficult for NK cells to find them Both T and NK cells are particularly efficient also in allogeneic settings such as the allogeneic haemopoietic stem cell transplantation (HSCT) to cure hematologic malignancies. Another particularly promising approach of cellular therapy is the use of genetically-engineered autologous T cells with chimeric antigen receptors (CAR) conferring specificity for antigens expressed by tumor cells. Also NK cells can be genetically engineered with CAR. Different from CAR-T, NK cells, equipped with an array of receptors involved in tumor cell recognition and killing, retain their ability to focus on neoplastic cells through such receptors, possibly making tumor escape mechanisms less effective. CAR NK therapy uses cells from donated duct blood, it can potentially be made before time and frozen for storage. This is different from some types of adoptive cellular therapy, like CAR T cell therapy, which can require a few weeks to prepare.In addition, they may be complementary to CAR-T cells. However, NK cell transfection resulted quite challenging. Thus, viral transduction display to possess variable levels of transgene expression and should compromise NK cell viability. Moreover, viral transduction requires dedicated facilities, high costs and lengthy preparation. Recently, electroporation of mRNA has been proposed as alternative of viral methods. Although the mRNA electroporation features a very low effect on the vitality and good efficacy, a relevant drawback are represented by the short-time expression of the transgene. Here we show a replacement procedure for NK cells transfection with plasmid DNA. With an efficiency of up to 50% and viability up to 65% it's the foremost efficient, non-viral, methodology existing thus far to deliver exogenous DNA into NK cells. By applying this method, we transfected exogenous CCR7 chemokine receptor conferring to the NK cells the ability to efficiently migrate in response to the chemokines. Moreover, the introduction of an anti-CD19 CAR confer to transfected NK cells a specific and powerful cytotoxicity against CD19+ leukemic cells. As a part of the innate system , natural killer cells do not have to acknowledge a selected abnormality (antigen) on viral-infected cells or cancer cells. This is in contrast to some functions of immune cells which result from immunologic memory (the kind of functions for which immunizations are designed). If a cell is not recognized as being a normal part of the body. These results illustrate a number of potential important applications of this novel transfection approach. Notably, the electroporation of DNA may allow to a non-integrating gene transfer with episomal vectors. Natural killer cells can also be used as a sort of immunoregulation. In this process, the NK cells regulate the function of the system by producing substances referred to as cytokines.

NK cells do not carry a T cell receptor restricted to a particular peptide epitope presented by major histocompatibility complex (MHC) molecules, but recognize stress ligands on cancer cells via activator receptors encoded by the germ line , which are counterbalanced by inhibitory receptors which are triggered by auto-MHC class I. Therefore, for adoptive cancer immunotherapy, HLA mismatched NK cells from healthy donors are preferred, which do not recognize tumor cells as "auto", thereby bypassing inhibitory signals. Since NK cells do not have a high risk of inducing graft versus host disease (GvHD), this approach is generally considered safe. Better understanding of NK cell biology, as well as the development of strategies to improve NK cell activity by blocking inhibitory receptor pathways or redirecting NK cells to tumors using bispecific antibodies or genetic modifications with CARs, has paved the way for many therapeutic approaches that are now actively pursued in a clinical setting.

Biography

Ingegnere T has conducted his studies needed for both the first grade degree (laurea triennale) and second grade degree (laurea specialistica) at Sapienza University of Rome. After his graduation he did his PhD at CNR in Rome. From 2014 to 2016 he spent his first Postdoc in the Immunology Lab of Patrizio Giacomini at IFO-Regina Elena Cancer Institute. Since April 2016 he is in the lab of Prof. Moretta as Postdoc in the Immunology Unit of Pediatric Hospital Bambino Gesù.

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