JORNAL DA SOCIEDADE DAS CIÊNCIAS MÉDICAS DE LISBOA

DOI: https://doi.org/10.57849/ulisboa.fm.jscml.0000011.2025

PFIZER RESEARCH AWARDS 2024

BASIC RESEARCH

Restoring tumor immunogenicity with dendritic cell reprogramming

Carlos-Filipe Pereira [1,2]

[1] CNC - Centre for Neuroscience and Cell Biology, University of Coimbra, Largo Marquês do Pombal 3004-517, Coimbra, Portugal.

[2] Molecular Medicine and Gene Therapy, Lund Stem Cell Centre, Wallenberg Centre for Molecular Medicine, Lund University, Lund, Sweden.

✉ filipe.pereira@cnc.uc.pt

Scientific Background

Cancer cells develop mechanisms to evade the immune system, including immunosuppression, intratumor heterogeneity, exclusion of immune cells from the tumor microenvironment, and downregulation of antigen presentation. Immunotherapies, such as immune checkpoint blockade (ICB) and adoptive T cell therapies, changed paradigms in cancer treatment. However, existing immunotherapies show limited efficacy in some patients. Notably, studies report that the success of ICB depends on tumor immunogenicity and antigen presentation to promote efficient CD8+ T cell priming.

Cellular reprogramming is the process of changing cell fate through epigenetic rewiring of a somatic cell type and the imposition of another desired cell identity, usually mediated by transcription factors (TFs). In cancer, cell fate reprogramming has been shown to lead to the disruption of oncogenic pathways and decreased tumorigenicity, but this strategy is limited by the requirement of reprogramming most cancer cells in the tumor. Ideally, a reprogramming-based strategy would be combined with an immunotherapeutic approach that has the potential to target immune activation while decreasing tumorigenicity. Immune activation greatly depends on type 1 conventional dendritic cells (cDC1), which cross-present tumor antigens to CD8+ T cells, prompting antitumor immunity. In fact, the presence of cDC1 in tumors correlates with better survival and response to immunotherapy. Overexpressing the three TFs PU.1, IRF8, and BATF3 (referred to as PIB) promotes direct reprogramming of mouse and human fibroblasts into immunogenic cDC1 (Rosa et al., 2018; 2022, Science Immunology). The current study (Zimmermannova et al., 2023,

Science Immunology) aimed at reprogramming cancer cells into professional, cDC1-like antigen-presenting cells (APCs) to counteract tumor immune evasion and induce tumor immunogenicity.

Research Project

This project aimed at testing the hypothesis that applying cDC1 reprogramming to cancer cells would efficiently reinstate immunogenicity. A lentiviral polycistronic vector encoding mouse or human PIB was used to evaluate whether direct cDC1 reprogramming could elicit antigen presentation in cancer cells. PIB overexpression induced surface expression of the pan-hematopoietic marker CD45 and the APC marker major histocompatibility complex (MHC)-II in mouse cells. Similarly, reprogramming progressed in human cells transduced with PIB, resulting in the expression of CD45 and the APC marker HLA-DR. The induced murine CD45+ MHC-II+ and human CD45+ HLA-DR+ populations were named tumor-APCs. CD45+ MHC-II+ cells showed surface expression of the cDC1-specific marker CLEC9A, and reprogrammed CD45+ HLA-DR+ cells showed CLEC9A, CD226, and CD11c expression, as well as cDC1-like morphological features. Transcriptional changes during reprogramming were evaluated by analyzing reprogrammed (CD45+ MHC-II+/CD45+ HLA-DR+) and partially reprogrammed (expressing either CD45 or MHC-II/HLA-DR) cells after 9 days of reprogramming. The transcriptome of reprogrammed cells mapped close to natural cDC1, irrespective of the species of origin. Particularly, tumor-APCs showed upregulation of genes related to antigen processing and presentation and immune interactions. Hence, the reprogramming process promoted transcriptional and phenotypic changes attributed to a cDC1 fate.

Further focusing on the transcriptional and epigenetic remodeling of cells undergoing cDC1 reprogramming, reprogrammed and partially reprogrammed populations were profiled along the reprogramming time course (days 3, 5, 7, and 9). Day 7 and day 9 populations mapped closer to peripheral blood cDC1, pointing to a gradual acquisition of a cDC1 transcriptional program. Analyzing differentially open chromatin regions revealed that epigenetic changes occurred mainly in

beginning of the process, from day 0 to day 3. cDC1 reprogramming was shown to be a stepwise process, imposing rapid epigenetic remodeling followed by gradual transcriptional changes.

Subsequently, Zimmermannova et al. addressed whether reprogrammed tumor-APCs processed and presented antigens and showed increased immunogenicity. Briefly, overexpressing PIB induced not only the expression of MHC class I and II but also the expression of costimulatory molecules, providing the critical signals for T-cell activation. This resulted in increased antigen presentation of endogenous antigens by tumor-APCs, as shown by immunopeptidomics and co-cultures with T cells recognizing model antigens endogenously expressed by cancer cells. In addition, cDC1 reprogramming promoted enhanced immune recognition and CD8+ T-cell killing of tumor-APCs.

To further analyze the functional properties of reprogrammed tumor-APCs, the study examined their ability to secrete proinflammatory cytokines, uptake antigens, and present them to CD8+ T cells. Reprogrammed cells acquired a cDC1 functional signature: they secreted cDC1-related cytokines and chemokines required for T-cell recruitment and activation, engulfed and processed proteins and dead cells, and cross-presented antigens to naïve CD8+ T cells. Notably, this system was not limited to cell lines; the demonstrated that patient-derived samples were permissive to cDC1 reprogramming at the single-cell level.

Since reprogramming cancer cells could potentially reduce tumorigenicity, transcriptional signatures were assessed during the reprogramming time course. Genes related to cell proliferation were downregulated, and tumor suppression genes were activated. Specifically, the downregulation of proliferation-related genes was common across most of the tested cell lines, highlighting an induction of cell cycle arrest with reprogramming. Additionally, both reprogrammed and partially reprogrammed cells showed slower cell division and a reduction in tumorigenic potential assessed both in vitro but also in vivo upon transplantation into immunodeficient mice.

Lastly, Zimmermannova et al. assessed the ability of in vitro-reprogrammed cells to promote antitumor immune responses in vivo. Injecting tumor-APCs intratumorally delayed tumor growth and extended survival in syngeneic mouse melanoma models. Moreover, combining the injection of tumor-APCs with ICB treatment fur-

ther reduced tumor growth and extended survival, emphasizing the synergy of cDC1 reprogramming and ICB in driving antitumor immunity in vivo.

The main findings of the current project include:

1. Demonstrated that the minimal cDC1 TF network elicits efficient cDC1 reprogramming in a broad panel of mouse and human cancer cell lines, as well as primary tumor samples.
2. Found that converting tumor cells into tumor-APCs, which exhibit characteristics similar to natural cDC1, promotes CD8+ T-cell activation, recognition, and the elimination of cancer cells. Reprogrammed tumor-APCs enabled presentation of endogenous tumor antigens on MHC-I, facilitating targeted CD8+ T-cell killing.
3. Showed that intratumoral injection of tumor-APCs elicited antitumor immunity, resulting in delayed tumor growth, extended survival, and improved response to ICB.

Implications for Future Research/Clinical Practice

Previously developed immune cell therapies and dendritic cell (DC)-based vaccines have a significant limitation, particularly the lack of methods to efficiently generate rare and specific immune subsets such as cDC1. Monocyte-derived DCs (moDCs) have been more frequently used in DC vaccines, since it is easier to isolate them from peripheral blood. However, moDC-based vaccines show limited clinical efficacy. Reprogramming cancer cells into cDC1, an immune subset crucial for antitumor immunity, shows great promise as a strategy to replenish this rare but potent immune compartment within the tumor microenvironment.

This study demonstrates that cDC1 reprogramming enforces antigen presentation, overcomes tumor evasion mechanisms, and enhances antitumor immunity. Importantly, cDC1 reprogramming was not restricted to specific cell types of origin; it progressed successfully in a wide range of cancer cell lines and primary tumor cells. The current project accelerated the preclinical development of an immunotherapy alternative that is feasibly applicable to all tumor types. Additionally, it could support the development of therapies based on in vitro tumor-infiltrating lymphocyte expansion and neoantigen discovery.

This study lays a strong foundation for future research into cancer-to-immune cell reprogramming and specification, particularly through in-depth molecular analyses. Specifically, the data generated—mRNA sequencing, single-cell mRNA sequencing, and ATAC-sequencing data at multiple time points and from various somatic cells—represents a valuable resource for understanding the gene dynamics underlying cDC1 reprogramming and specification. Zimmermannova et al. have advanced cDC1 reprogramming toward clinical application. Moreover, these extensive datasets will help answer critical questions, such as why some cancer cells reprogram more efficiently than others and how the cDC1 antigen processing and presentation machinery influences the quality of peptides displayed on the surface of reprogrammed cells.

In summary, converting tumor cells into immune cells that excel in antigen presentation represents an innovative therapeutic alternative, paving the way for the development of a novel cancer immunotherapy modality. This approach reverses the tumorigenic potential of cancer while promoting antigen presentation to enhance the antitumor activity of $CD8^+$ T cells. Building on this study, efforts to reprogram cancer cells in situ using adenoviral vectors are already spearheading the development of the next generation of cancer immunotherapies (Ascic et al., 2024, Science).

PAPER DISCUSSED

Zimmermannova O, Ferreira AG, Ascic E, et al. Restoring tumor immunogenicity with dendritic cell reprogramming. *Sci Immunol.* 2023;8(85):eadd4817. doi:10.1126/sciimmunol.add4817

Know more.
See the promotional video.