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2022

Document Version: Publisher's PDF, also known as Version of record

Link to publication

Citation for published version (APA):

Ascic, E., Barros Ferreira, A. G., Benonisson, H., Kurochkin, I., Nascimento Caiado, I. M., Zimmermannova, O., Alves, R., Fiúza Rosa, F., Pires, C., & Pereira, F. (2022). *Harnessing Dendritic Cell Reprogramming to Elucidate Mechanisms of Tumor Immunity*. Poster session presented at Lund Stem Cell Center Retreat, Båstad, Sweden.

Total number of authors: 10

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Harnessing Dendritic Cell Reprogramming to Elucidate Mechanisms of Tumor Immunity





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Abstract

The presence of conventional dendritic cells type 1 (cDC1) in the tumor correlates with positive treatment outcome. The ability to cross-present neoantigens and prime protective CD8+ T-cell responses, makes cDC1s central for tumor immunity. However, in tumors cDC1 are rare and often functionally impaired [1]. Our group reported that overexpression of the transcription factors PU.1, IRF8 and BATF3 (PIB) converts mouse and human fibroblasts into cross-presenting cDC1-like cells [2, 3]. We employed the minimal gene regulatory network of highly immunogenic cDC1 and restored the immunogenicity of low immunogenic lung cancer and melanoma cell lines by reprogramming into professional tumor antigen presenting cells (tumor-APCs) [4].

Here, we report that upon transduction with PIB, 23 solid syngeneic cancer lines initiate reprogramming into cDC1-like cells expressing CD45 and MHC-II at efficiencies ranging from 0.5-57.7%. Functionally, PIB overexpression endows tumor cells with the capacity to cross-present exogenous antigen and prime naïve CD8+ T-cells. Adoptive transfer of ovalbumin cross-presenting B16 tumor-APCs into established ovalbumin expressing B16 tumors (B16-OVA) elicits tumor growth control and extends animal survival. Treated animals show a systemic antigen-specific T cell response against ovalbumin and endogenous tumor-associated antigen MuLV p15E. Intratumoral injection of reprogrammed B2905 and LLC into tumors shows differential response, correlating with their cross-presentation capacity.



This approach combines cDC1 antigen cross-presentation abilities with the generation of tumor antigens. The induction of a cDC1 identity in tumor cells sets in motion T cell responses in vitro and in vivo. In the future of this project, dendritic cell reprogramming will be object in a 2-cell CRISPR/Cas9 screen using induced cDC1-like tumor cells and reporter T-cells to explore mechanistically cross-presentation regulators. The generation of cross-presenting tumor-APCs will be also used to map and characterize presented and cross-presented neoantigens. Finally, dendritic cell reprogramming of tumor cells will be explored in vivo by replenishing cDC1 within the tumor microenvironment through in vivo reprogramming. Ultimately, this project will provide insight into mechanisms of cross-presentation and pave the way for the development of novel cDC1-centric therapies.

Results







Fig 2. PU.1, IRF8 and BATF3 Endow Mouse Cancer Cells with cDC1 Function Cross-Presentation. Tumour-APCs at reprogramming day 8 were pulsed with ovalbumin antigen and polyIC before coculture with ovalbumin-specific naive CD8+ T cells (OT-I) for 3 days. OT-I cells were membranelabelled with CellTrace violet (CTV) to measure proliferation and stained for activation marker CD44. A) Quantification of CD44+ proliferative OT-I CD8+ T cells after co-coculture. (n=4) B) Representative flow cytometry plot of CD8+ T cell proliferation (CTV) and activation (CD44) after co-culture with PIB-eGFP transduced (lower) and control eGFP transduced (upper) B2905 cells.



Fig 3. Adoptive Transfer of Cross-Presenting Tumor-APC Elicits Anti-Tumor Immune Response. A) PIB and empty vector (MCS) control transduced B16 cells were pulsed with ovalbumin antigen and polyIC and injected into established B16-OVA tumors at day 7, 10 and 13. B, C) Tumor growth (B, n=6) and survival (C, n=16) of B16-OVA tumor bearing mice injected with B16 cells transduced with PIB or MCS and pulsed with ovalbumin and polyIC or injected with PBS. ****p<0.0001



Fig 5. Anti-Tumor Immune Response Elicited by **Tumor-APCs Correlates with Cross-Presentation** Capacity. WT tumors were established and injected with polyIC stimulated tumor cells transduced with PIB or MCS or injected with PBS at day 10 post-tumor establishment. A) Tumor growth in mice injected with B2905 tumor cells and tumor-APCs or empty vector (MCS) control transduced cells or PBS (n=3-4). B) Tumor growth in mice injected with LLC tumor cells and tumor-APCs or empty vector (MCS) control transduced



Fig 4. Tumor APC elicit systemic tumor antigen-specific T cell response. Blood of B16-OVA tumor bearing mice was collected after 3 intratumoral injections with B16 cells transduced with PIB or MCS pulsed with ovalbumin and polyIC. A) Representative flow cytometry plot of blood T cells stained with OVA peptide SIINFEKL (256-264) tetramer. B) Quantification of OVA-specific T cells from blood of treated mice (n=10). C) Representative flow cytometry plot of blood T cells stained with murine leukemia virus peptide p15E tetramer. D) Quantification of p15E-specific T cells from blood of treated mice (n=10).

Conclusions and Outlook

PU.1, IRF8 and BATF3 induce a tumor-APC phenotype in all 23 tested syngeneic solid cancer models

Future Directions



Dendritic cell reprogramming endows murine cancer cells with cDC1 hallmark function cross-presentation

Cross-presenting B16 melanoma tumor-APCs elicit tumor growth control, extend animal survival and stimulate systemic antigen-specific T cell responses

Adoptive transfer of B2905 and LLC tumor APC into tumors indicate correlation of treatment response with cross-presentation capacity



Acknowledgements



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