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Interactions of Melanoma Associated Fibroblasts with Macrophages and Gamma Delta T cells

PhD dissertation

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1. Introduction

The investigation of mechanisms involved in malignant tumor formation and the search for therapeutic targets initially focused on genetic, molecular biological and metabolic events occurring within the transformed cell itself. However, according to the latest scientific results, numerous non-malignant cells within the tumor microenvironment can also contribute to the proliferation of cancer cells and the growth of tumors. The non-malignant cells in the tumor microenvironment include stromal cells and various immune cells. Stromal cells including tumor-associated fibroblasts, tumor-associated adipocytes, tumor endothelial cells, and pericytes, while immune cells consist of tumor-associated macrophages, tumor-associated neutrophil cells, NK cells, dendritic cells, myeloid-derived suppressor cells, and various T and B cell populations [1].

The most lethal type of skin cancer is melanoma, and its incidence is increasing every year. In melanoma, tumor-associated fibroblasts, known as melanoma-associated fibroblasts (MAFs), are present in the tumor microenvironment. The structural proteins produced by MAFs provide spatial support structures and a protective network for melanoma cells. MAFs may originate from various possible precursors, such as resident fibroblasts in the tumor environment, bone marrow-derived

mesenchymal stromal cells, hematopoietic stem cells, endothelial cells, or local epithelial cells.

Our research group previously demonstrated that MAFs share functional similarities with mesenchymal stromal cells, such as inhibiting the activity of cytotoxic T lymphocytes. Additionally, we analyzed the differentiation potential, immunosuppressive effects, and colocalization of MAFs with other immune cells in the tumor environment. Based on these results during my individual research, I investigated the immunomodulatory effects of MAFs on primary human monocyte-derived macrophages. Furthermore, following the identification of the inhibitory effect of MAFs on cytotoxic $CD8^+$ T-cell activity, my research work aimed to explore how immunosuppressive MAFs could be eliminated. I explored the possibility of apoptosis induction by the cytotoxic effect of an unconventional T cell population: γδ T-cells.

γδ T cells, in comparison with the conventional αβ T cells do not recognize antigens of peptide nature bound to the target cells' surface major histocompatibility complexes (MHC) but their antigen recognition is performed in an MHC-independent manner. Currently, therapeutic approaches employing γδ T cells for solid cancer treatment resulted in mixed results. Therefore, my research focused on understanding the biology of γδ T cells in melanoma, emphasizing the interaction between γδ T cells and MAFs

2. Research objectives

A significant part of current research on melanoma primarily targets tumor cells. In contrast, the focus of my doctoral thesis was not the study of tumor cells but rather on investigating other cells within the tumor microenvironment. Specifically, I analyzed the interaction between MAFs (cells forming the tumor stroma) and certain immune cell populations. In the beginning, I examined how MAFs interact with primary macrophages, considering the immunomodulatory and immunosuppressive effects of MAFs. Subsequently, I explored the interaction between MAFs and a unique immune cell population known for its specific anti-tumor effects—the γδ T cells. The goal was to determine whether γδ T cells could potentially eliminate immunosuppressive MAFs. To guide my research, I established the following detailed objective:

1. One of the main characteristics of mesenchymal stromal cells is their immunmodulatory property. Since MAFs exhibit functional similarities with MSCs, such as inhibiting the activity of cytotoxic $CD8^+$ T cells, initially I sought to answer the question of how MAFs influence the immune functions of macrophages. My first objective was to establish an *in vitro* differentiation assay using primary monocytes to generate M1 and M2 macrophages for the examination of immunomodulation.

2. For my second objective, I planned to analyze M1 and M2 macrophages differentiated from monocytes in a coculture assay. In this assay, macrophages would be co-cultured with previously isolated MAF cells, and after LPS stimulation of the coculture, the amount of the immunosuppressive IL-10 cytokine would be measured in the cell supernatant.

3. The application of γδ T cells is a promising area in cellular therapy research and development. Given the extensive investigation of the interaction between tumor cells and γδ T cells in various cancer types, my focus shifted to examining the interaction between γδ T cells and MAFs. The aim was to determine whether immunosuppressive MAFs could be eliminated by anti-tumor γδ T cells. For this purpose, my third outlined objective was to expand and isolate γδ T cells from peripheral blood.

4. Furthermore, my fourth objective was to determine - using isolated γδ T cells and MAFs - whether it is possible to create *in vitro* conditions under which γδ T cells can induce apoptosis in MAFs.

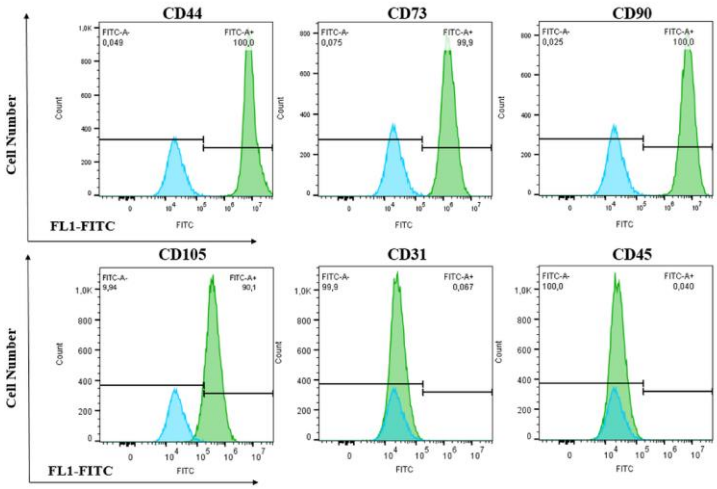
5. γδ T cells posses MHC-independent antigen recognition characteristic. My fith objective was to investigate whether γδ T cells from melanoma patients are able to induce apoptosis in MAFs matching the donor.

6. For my sixth objective, I planned to explore the molecules that play a role in the mechanism of MAF apoptosis induced by γδ T cells in the *in vitro* assays

3. Novel Scientific Results

Thesis 1.a: Melanoma associated fibroblasts show functional similarities with MSCs. A qualitative indicator of this is the expression of traditional stem cell markers on the surface of MAF cells.

Hypothesizing the functional similarities between MAFs and MSCs I analyzed the cell surface expression of the markers below defined by the International Society for Cell and Gene Therapy (ISCT) (***1. Figure***). CD44, CD73, CD90 and CD105 markers showed positivity above 90%, while the expression of platelet endothelial cell adhesion molecule-1 (PECAM-1) CD31 and to the common leukocyte antigen CD45 was below 1 %.



1. Figure: MSC-like marker expression of MAFs. Histograms show cell surface antigen expression of MAFs (green) compared to unstained control (blue), n = 3.

Thesis 1.b: MAFs have the ability to modulate the immune response of primary human macrophage cells. We can observe the anti-inflammatory changes in immunomodulation by measuring the prognostically significant IL-10 cytokine in in vitro cocultures of MAFs and macrophages

Primary monocyte isolation was performed from the heterogenous immune cell population of peripheral blood mononuclear cells (PBMCs) of healthy individuals. By the presence of the CD14 marker on monocytes they can be specifically selected and used as a homogenous cell population.

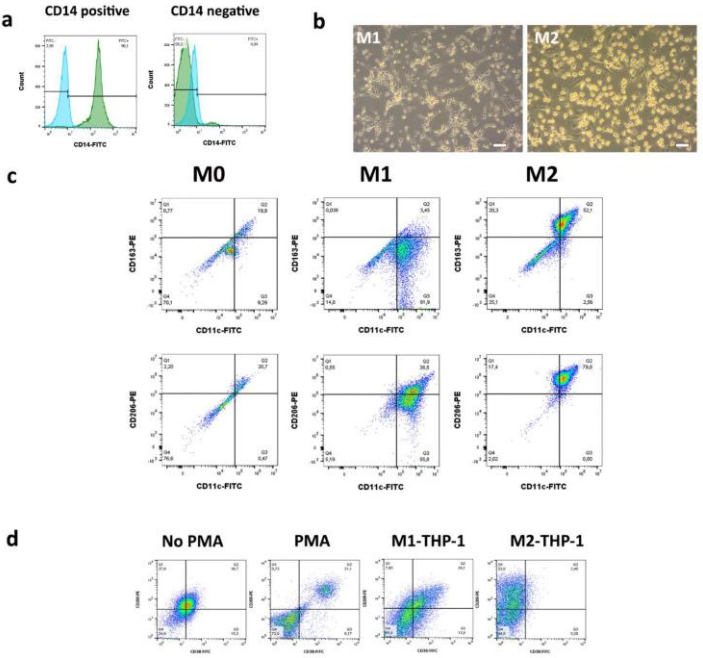
Subsequently, I polarized the monocyte cells into M1 and M2 phenotypes in various cytokine environments. The differentiation protocol was established using the THP-1 human monocyte cell line before applying it to primary cells.

After nine days of differentiation, M1 macrophages exhibited a morphology characterized by a round cell body and longer, thinner cytoplasmic extensions. In contrast, the morphology of M2 macrophages showed shorter, thicker cytoplasmic extensions (***2. Figure*** part b).

In terms of cell surface markers, the M1-specific CD11c marker was present in over 85% of the macrophages differentiated in the M1 direction by us. However, M2 macrophages also showed positivity above 50% for this marker. (***2. Figure*** part c). The M2-specific CD163 marker showed positivity above 70%, while the also M2-specific CD206 marker was present in over 90% of macrophages differentiated in the M2 direction (***2. Figure*** part c). To M2 macrophage markers, it is also true that to a lesser extent, they were present on macrophages differentiated in the M1 direction. This result suggests that under the applied experimental conditions (cytokines), the M1-M2 immunophenotype in macrophages cannot be clearly distinguished into two homogeneous groups. Mixed immunophenotypic groups of M1-M2 develop, and their functional properties are presumably determined by the specific cytokine environment

Similarly, for THP-1 macrophage cells, we observed a similar pattern in terms of cell surface marker expression: the M1-specific marker was

present in about 35% of M1-likeTHP-1s, while it was around 2% on M2-like THP-1 cells. (***2. Figure*** part d). The M2-specific CD209 marker exhibited positivity above 50% on M2-THP-1 cells, but it was also present in about 30% of M1-like THP-1 cells (***2. Figure*** part d).

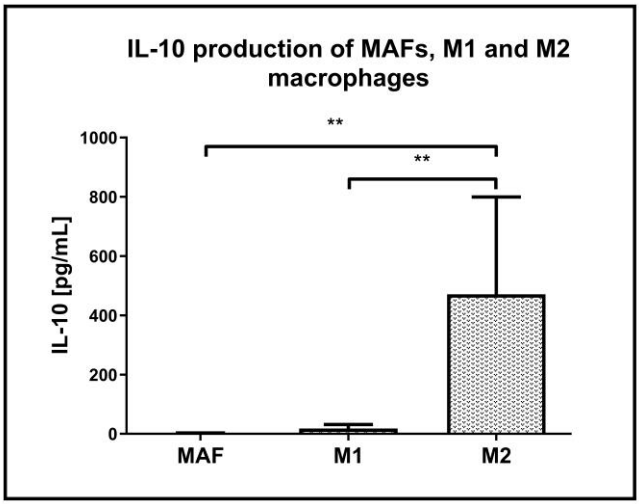


2. Figure: Characterization of *in vitro* differentiated macrophages (a) CD14-positive (left) and -negative (right) PBMC fractions after magnetic bead separation. (b) Morphology of monocyte-derived M1 (left) and M2 (right) macrophages; scale bar = 20 µm. (c) CD163, CD206, CD11c cell surface marker expression in M0, M1, and M2-like macrophages. (d) CD38 and CD209 cell surface marker expression in non-differentiated THP-1

cells without PMA stimulation or PMA-stimulated and M1- and M2-like differentiated THP-1 cells

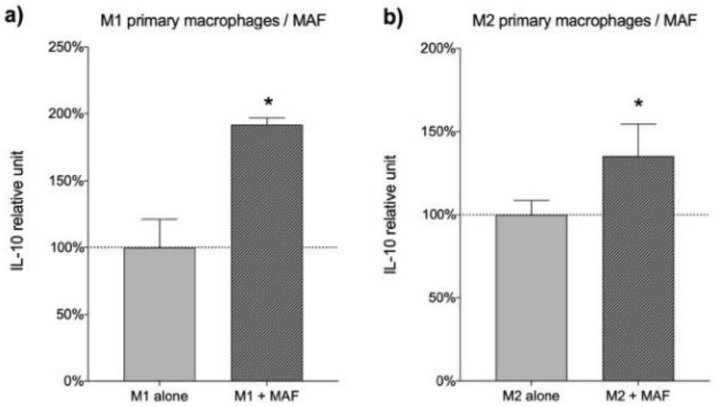
The direction of the inflammatory M1 and M2 immunophenotypes of monocytes derived from human peripheral blood could be distinguished not only by morphology, and cell surface marker expression but their IL-10 cytokine secretion as well.

M2 macrophages with immunosuppressive properties, exhibited significantly higher IL-10 production compared to M1 macrophage cells (***3. Figure***). MAFs, on their own, do not produce IL-10. Therefore, I further investigated how they can modify macrophage properties through the promotion of immunosuppressive IL-10 production.



3. Figure: IL-10 secretion of M1 and M2 immunphenotyped, differentiated macrophages and MAFs. (n=3). Error bars represent mean values and ± standard deviation (SD) *** $p < 0.001$

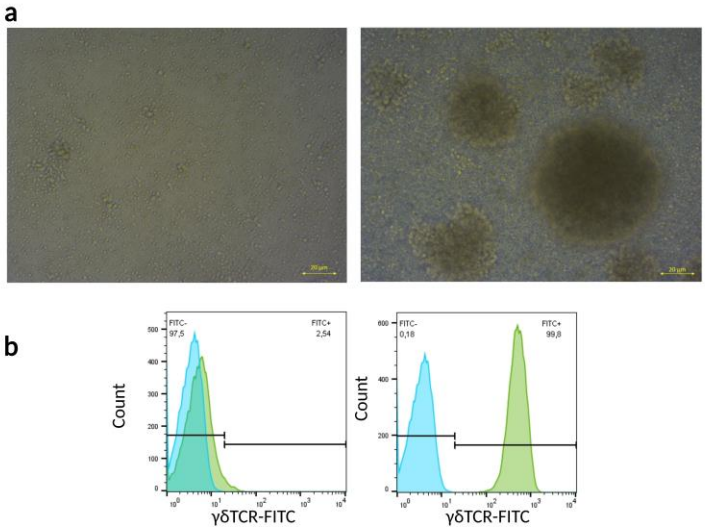
The presence of MAFs resulted in a significant increase in IL-10 secretion by macrophages (***4. Figure***), including polarized THP-1 cells and M1 and M2-type macrophages differentiated from primary monocytes as well. Based on these results, I confirmed that MAFs influence not only other immune cells but also macrophages by enhancing their immunosuppressive IL-10 production.



4. Figure Effect of MAFs on IL-10 secretion in primary macrophages from healthy donors. Relative IL-10 concentration of MAF/M1-like (a) and M2-like (b) differentiated primary macrophage co-culture compared to monoculture, n = 3. Error bars represent means ± s.e.m. * $p < 0.05$

Thesis 2a: The anti-tumor T lymphocte subpopulation: γδ T cells can be isolated from peripheral blood and under in vitro circumstances they are able to induce apoptosis in MAFs.

Due to the low percentage of peripheral blood γδ T cells (1-5%), I increased their ratio in my *in vitro* experiments by adding zoledronic acid, following a previously published protocol [2], [3]. After the initial zoledronic acid stimulation, on the seventh day of proliferation I magnetically separated the PBMCs, and analyzed the two fractions of the separation process using flow cytometry. The fraction bound-and then --eluted by the magnetic column contained homogeneous γδ T cells (with a cell population purity of around 99%) at each separation step during my experiments (***5. Figure*** part b).



5. Figure: ZA and IL-2 mediated PBMC proliferation and magnetic separation of γδ T cells. (a) Morphology of unstimulated (left) and ZA- and IL-2-stimulated PBMCs 7 days post-stimulus (right). Scale bar = 20 µm. (b) Representative flow cytometry histograms of γδTCR-negative (left) and -positive (right) PBMC fractions after magnetic separation. Blue: unlabeled cells, green: γδTCR-FITC-labeled cells. Separation purity (n = 4): 99.2 ± 0.43.

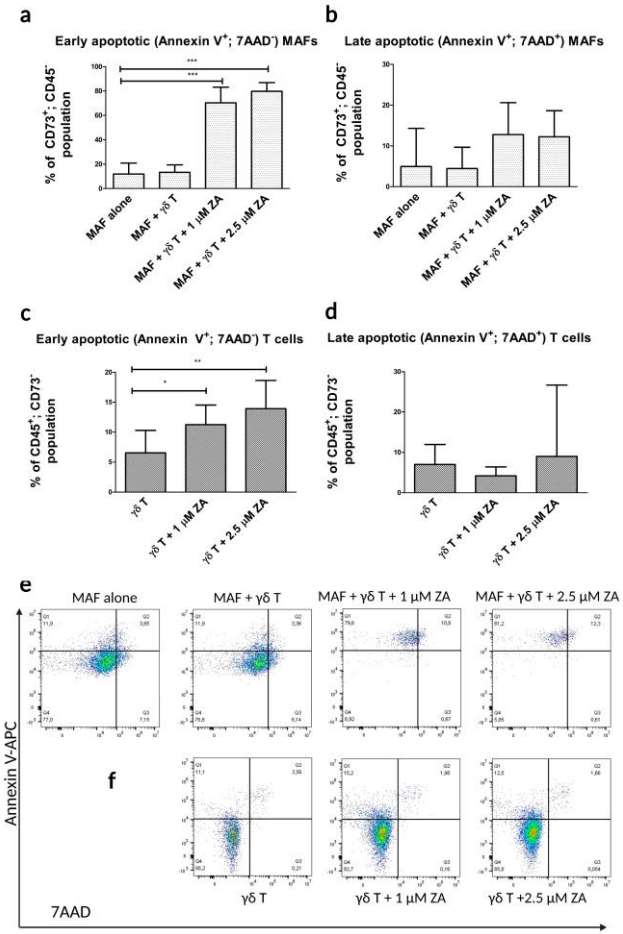
I explored whether zoledronic acid, necessary for the proliferation of γδ T cells, alone is sufficient in a pre-stimulus state to exert the cytotoxic effect of γδ T cells on MAFs. My results indicated that in the co-culture of γδ T cells and MAFs without stimulation, the apoptotic populations were similar to those in the sole MAF monocultures (**6. Figure** part a), and further zoledronic acid stimulus is needed to enhance their cytotoxic activity.

Through preliminary experiments I optimized the duration of the co-culture and the concentration of exogenously added zoledronic acid. Additionally, in a control experiment, I examined the effect of zoledronic acid on MAF cells to ensure that the increased apoptosis is solely attributable to γδ T cells and not to the the zoledronic acid.

Following this, I performed the optimized five-day apoptosis assay on γδ T cell-MAF co-cultures using MAF cells derived from various donors (**6. Figure**).

In this case, γδ T cells were isolated from a single healthy donor to ensure that the differences in apoptosis among MAFs were not influenced by variations in γδ T cell donors. In each assay performed at a specific time point, I consistently utilized γδ T cells from a single donor, allowing donor differences to be seen only by MAF members of the co-culture.

From the various zoledronic acid stimulus concentrations in the experimental conditions, I only kept concentrations of 1 μM and 2.5 μM. The early apoptotic populations were avaragely over 65% for 1 μM and reached nearly 80% for 2.5 μM zoledronic acid (**6. Figure** part a). The late apoptotic populations were around 10% (**6. Figure** part b). I also examined the apoptosis of γδ T cells alongside MAFs, analyzing both early and late apoptotic populations, each of which was below 10% (**6. Figure** part c and d) In the co-cultures stimulated by zoledronic acid the early apoptotic populations of γδ T cells were significantly higher compared to the non-stimulated control.



6. Figure: Flow cytometry apoptosis assays in γδ T cell and MAF co-cultures using the formerly optimized conditions (5 days of co-culture with 1 µM and 2.5 µM ZA-stimulated cells. (a) Percentages of early apoptotic (Annexin V^+, $7AAD^-$) and (b) late apoptotic (Annexin V^+, $7AAD^+$) populations of MAFs ($CD73^+$; $CD45^-$). n = 10. Control: MAF mono-culture without γδ T cells. (c) Percentages of early apoptotic and (d) late

apoptotic populations of γδ T cells ($CD45^+$; $CD73^-$) after 5 days of co-culture with MAFs. Control: co-culture with unstimulated γδ T cells. (e) Flow-cytometric dot plots of apoptotic fibroblast populations. (f) Flow-cytometric dot plots of apoptotic γδ T cells. Error bars represent means ± SD. * $p < 0.05$ ** $p < 0.01$ and *** $p < 0.001$.

Thesis 2b: γδ T cells possess MHC-independent target recognition. As confirmation, peripheral γδ T cells from patients with a history of melanoma are also capable of inducing apoptosis in MAFs.

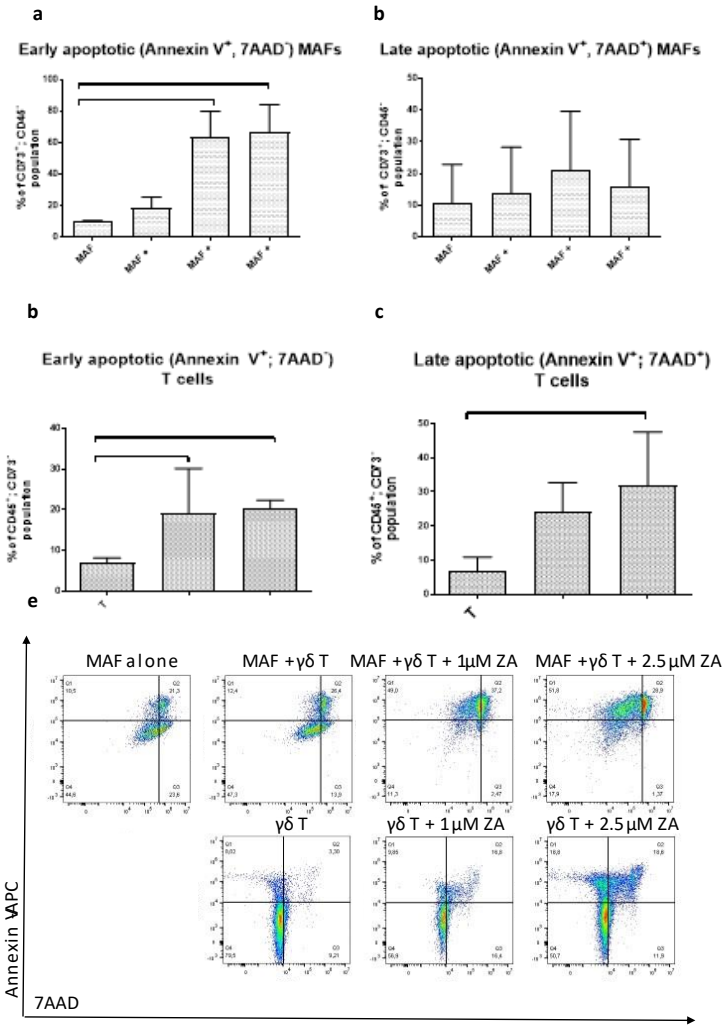
Due to the MHC-independent ligand recognition properties of γδ T cells, they can potentially serve as tools for autologous and allogeneic cell therapies. Therefore, in the majority of my experiments, I utilized γδ T cells derived from healthy individuals. However, considering the possibility of autologous γδ T cell therapy as well as zoledronic acid therapy, I intended to show the importance of the examination of the cytotoxic effects of γδ T cells from melanoma patients. For the repeated apoptosis assay in γδ T cell-MAF co-cultures, I used previously isolated MAF cells from two donors and stored them in our biobank. For these donors, I isolated PBMC sas well from venous blood and expanded then separated γδ T cells with the assistance of zoledronic acid.

Without stimulus, γδ T cells did not induce apoptosis in MAFs; however, following stimulation with 1 and 2.5 μM zoledronic acid and IL-2, significantly higher early apoptotic populations were present (**7. Figure** part a).

With this experimental setup, I demonstrated that the cytotoxic effect of γδ T cells is effective in the case of MHC-matched target cells, thus occurring in an MHC-independent manner as well.

In the γδ T cell-MAF co-culture derived from melanoma patients, γδ T cells exhibited a higher degree of apoptosis compared to the γδ T cells from healthy volunteers in MAF co-cultures. During the isolation of γδ T

cells from proliferated PBMCs, I observed that a fewer number of γδ T cells could be obtained. As the expansive capacity of γδ T cells is highly dependent on the individual characteristics of donors. Due to the limited number of γδ T cells from melanoma patients, we have not been able to confirm this observation with further investigations at this time [3], [4].



7. Figure: Melanoma patient derived γδ T cell and MAF interaction. In the experimental setup of the figure biobanked MAF cells were paired with

each patients' own γδ T cells in the apoptosis assay. (a) Early (Annexin V^+; $7AAD^-$) and (b) late apoptotic MAF (Annexin V^+ 7AAD+) [$CD73^+$, $CD45^-$] populations. (c) Early (Annexin V^+; $7AAD^-$) and (d) late (Annexin V^+ $7AAD^+$) apoptotic γδ T cell [$CD45^+$, $CD73^-$] populations. (e) Representative flow cytometry dot plot of apoptotic fibroblasts and γδ T cells

Thesis 2c: In the interaction between γδ T cells and MAFs, similar molecules are involved as those identified in previous studies on interactions between tumor cells and γδ T cells.

To investigate the molecular background of the cytotoxic effect of γδ T cells on MAFs, I analyzed the target molecules of the main receptors of γδ T cells (γδ TCR, NKG2D) at the mRNA level in MAFs using reverse transcription and quantitative PCR. Subsequently, among the molecules examined at the mRNA level for gene expression, my goal was to analyze the BTN3A1 γδ TCR target at the protein level. I also aimed to explore the extent to which apoptosis is influenced in cellular co-cultures where the level of BTN3A1 protein is low and where it is high.

At the protein level, SK-MEL-28 melanoma cells expressed the BTN3A1 molecule at the greatest level with the analyzed population showing over 50% positivity compared to the unlabeled SK-MEL-28 control (**8. Figure** part a). MAFs avaragely showed positivity above 10% compared to an unlabeled MAF sample (**8. Figure** part a). Thus both cell type resulted in significantly higher BTN3A1 expression compared to NDF cells, where BTN3A1 was barely detectable.

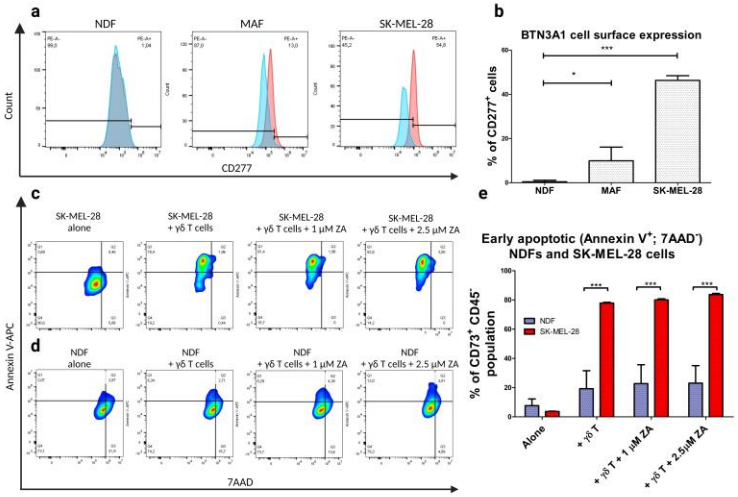
Based on flow cytometry measurements of BTN3A1 on the cell surface, NDFs were categorized as having very low expression, while the SK-MEL-28 melanoma cell line was classified as having high BTN3A1 expression. We repeated the five-day flow cytometry apoptosis assay with γδ T cells and SK-MEL-28 or NDF cells, where the co-culture conditions matched those of the previous conditions. In the case of NDF cells non-stimulated

γδ T cells did not cause significant increase in the early apoptotic populations (Annexin V^+, $7AAD^-$) (**8. Figure** part e). Furthermore, zoledronic acid in the concentration of 1 and 2,5 μM and IL-2 in a concentration of 100 IU/mL did not induce apoptosis in the case of NDF cells in a higher amount as γδ T cells without zoledronic acid treatment.

Compared to apoptosis assays with MAFs, in the SK-MEL-28-γδ T co-culture, the early apoptotic population was significantly higher in the unstimulated γδ T cell-SK-MEL-28 experimental setup compared to the SK-MEL-28 only control (**8. Figure** part e).

Furthermore, 1 and 2.5 μM zoledronic acid did not increase the early apoptotic populations in SK-MEL-28 cells; instead, it similarly resulted in an 80% apoptotic cell population. In the MAF-γδ T cell co-culture (**6. Figure** and **7. Figure**) I did not observe that the addition of zoledronic acid was negligible, so we concluded that in the case of cells with high BTN3A1 expression, γδ T cells have potent cytotoxic effects.

Since the γδ T cell cytotoxic activity did not have a significant effect on NDF cells with very low BTN3A1 levels, whereas it did on MAFs with higher BTN3A1 expression and the melanoma cell line (SK-MEL-28), it confirmed that the higher the cell surface expression of the BTN3A1 molecule on the target cell, the greater the extent of apoptosis.



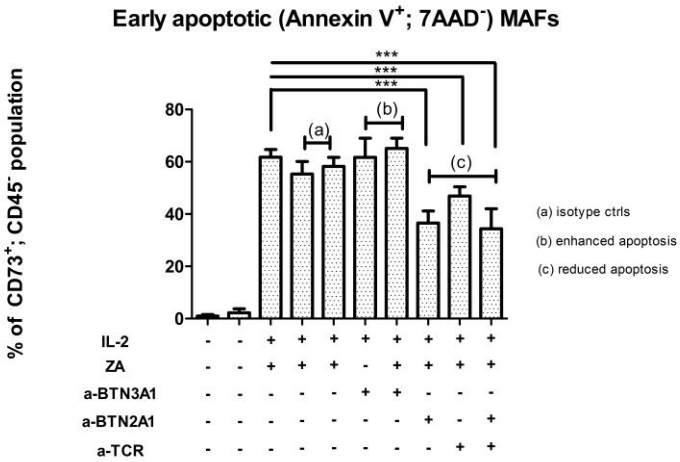
8. Figure: The surface expression of BTN3A1 and γδ-T-cell-induced apoptosis in fibroblasts and SK-MEL-28 cells. (a) Representative histograms of BTN3A1 expression in NDFs, MAFs, and SK-MEL-28 cells. (b) BTN3A1 expression on fibroblasts and the SK-MEL-28 cell line. (c) Representative density plots of early apoptotic (Annexin V^+; $7AAD^-$) populations in the SK-MEL-28 cell line. (d) Representative density plots of early apoptotic (Annexin V^+; $7AAD^-$) populations in NDFs. (e) Percentage of early apoptotic populations of NDFs and the SK-MEL-8 cell line [$CD73^+$ $CD45^-$] after 5 days of co-culture with γδ T cells. Control: co-culture with unstimulated γδ T cells. (d) Representative histograms of BTN3A1 expression in NDFs (n=3), MAFs (n=3), and the SK-MEL-28 cell line. Error bars represent means ± SD .*$p < 0.05$ **$p < 0.01$ and ***$p < 0.001$.

To obtain a more detailed, molecular-level understanding of the interaction between γδ T cells and MAFs, I modified and repeated the original apoptosis assays. Based on my hypothesis, the molecules previously identified in the interaction between γδ T cells and tumor cells were presumed to contribute to the apoptosis of MAFs [5], [6].

In addition to the γδ T cell-MAF conditions without stimulation and those stimulated with 1-2.5 μM zoledronic acid, I introduced experimental setups where the co-culture included butyrophilins and/or antibodies targeting the γδ TCR. I applied a stimulating (enhancing γδ T cells' cytotoxic effect) anti-BTN3A1 antibody (clone 20.1) at a concentration of 1 μg/mL, both without zoledronic acid and in the presence of zoledronic acid. The aim was to investigate whether it could increase the early apoptotic population in MAFs [7]–[9].

The inhibition of apoptosis with anti-TCRγδ and anti-BTN2A1 antibodies, along with my previous findings regarding BTN3A1 cell surface presence, supported that the γδTCR–BTN2A1-BTN3A1 axis is the primary factor in triggering apoptosis.

The fact that the inhibition of apoptosis with anti-TCRγδ and anti-BTN2A1 antibodies resulted in only partial reduction indicates that other molecules (e.g., NKG2D-MICA/MICB/ULBP interaction) may also play a role in this interaction.



9. Figure: Early apoptotic populations of MAFs with additional conditions, including BTN2A1 inhibition by anti-BTN2A1 antibody, γδ T cell receptor blocking with anti-TCR γδ (Clone B1) antibody, and BTN3A1 stimulation by CD277 monoclonal antibody (clone 20.1) and mouse IgG isotype control. Error bars represent means ± SD. * $p < 0.05$ *** $p < 0.001$.

Based on my experimental results, it can be hypothesized that similar to the process occurring in tumor cells, zoledronic acid contributes to the accumulation of IPP in MAFs as well. Subsequently, IPP binds to the intracellular part of the BTN3A1 molecule, inducing a conformational change that allows it to interact with BTN2A1 through its intracellular B30.2 domains. This active conformation facilitates the binding of the γδ T cell Vγ9 chain (between the CDR2 and CDR3 regions) to BTN2A1, thereby activating the cytotoxic function of γδ T cells and enabling them to induce apoptosis in MAFs. It is important to note that the binding

partner of BTN3A1 on the γδ T cell is not known, but there is speculation about a ligand that connects the γδ TCR CDR3 region with BTN3A1.

4. Conclusions

In the melanoma tumor microenvironment, tumor-associated macrophages (MAFs) play a prominent role in tumor growth, influencing intratumoral immune mechanisms and local metastasis formation [10]. As MAFs are known to inhibit $CD8^+$ T cells and NK cells within the tumor, my research focused on their interaction with another immune cell population: macrophages. The classical M1-M2 macrophage polarization is a well-known phenomenon [11]. However, in the tumor microenvironment, a homogeneous population of these two phenotypes is rarely observed. Instead, a mixture of heterogeneous macrophage populations are present, transitioning continuously between M1 and M2 states. Investigating the cytokine production and combined immunosuppressive effects of these two macrophage types poses a challenge, but levels of certain cytokine types can indicate how much a tumor influences the anti-tumor immune response. IL-10 holds particular significance among immunosuppressive cytokines [12]. In various cancers, tumor-associated macrophages (TAMs) producing IL-10 correlate with unfavorable prognosis and low survival rates [13], [14]. Furthermore, intra-tumoral IL-10 secretion is linked to the primary melanoma's metastatic potential [13], [15]. Given the immunosuppressive similarities between MAFs and MSCs, my investigations aimed to answer how MAFs influence the IL-10 secretion of macrophages.

Additionally, my research goal was to answer the question of whether zoledronic acid, a drug accepted for over fifteen years and also suitable for *ex vivo* proliferation of γδ T cells, can be applied in an *in vitro* model for the activation of γδ T cells and elimination of MAFs. In my experiments, the apoptosis of MAFs was demonstrated through γδ T cells, and I identified butyrophilin molecules in MAFs that were previously shown in the interaction between γδ T cells and tumor cells.

I believe that the data from my research contribute to a more detailed understanding of the melanoma tumor microenvironment and may initiate further studies in both investigating the immunosuppressive effects of MAFs and exploring the therapeutic applications of γδ T cells. Moreover, the development of these strategies holds promise for the treatment of aggressive solid tumors.

5. Publications related to the thesis

Uğur Çakır, Anna Hajdara, Balázs Széky, Balázs Mayer, Sarolta Kárpáti, Éva Mezey, Pálma Silló, Gergely Szakács, András Füredi, Zoltán Pós, Barbara Érsek, Miklós Sárdy, Krisztián Németh (2021) Mesenchymal-Stromal Cell-like Melanoma-Associated Fibroblasts Increase IL-10 Production by Macrophages in a Cyclooxygenase/Indoleamine 2,3-Dioxygenase-Dependent Manner, Cancers, 13 : 24 Paper: 6173 , 22 p (IF: 5,2)

Anna Hajdara, Uğur Çakır, Barbara Érsek, Pálma Silló, Balázs Széky, Gábor Barna, Shaaban Faqi, Miklós Gyöngy, Sarolta Kárpáti, Mayer Balázs (2023) Targeting Melanoma-Associated Fibroblasts (MAFs) with Activated γδ (Vδ2) T Cells: An In Vitro Cytotoxicity Model, International Journal of Molecular Sciences, 24 : 16 p. 12893 (IF: 5,6)

Anna Hajdara, Uğur Çakır, Barbara Érsek, Pálma Silló, Balázs Széky, Gábor Barna, Shaaban Faqi, Sarolta Kárpáti, Krisztián Németh and Balázs Mayer (2023) Zoledronic acid activated Gamma Delta T cells Induce Apoptosis in Melanoma-Associated Fibroblasts; Hungarian Society for Immunology 52nd. Itinerary congress, Kecskemét

6. Further publications of the author

Jiaqiang Ren, Gergely Szombath, Lynn Vitale-Cross, David Stroncek F., Pamela Robey G., Anna Hajdara, Ildikó Szalayova, Balázs Mayer, Daniel Martin, Éva Mezey, Krisztián Németh (2023) The Potential Use of THP-1, a Monocytic Leukemia Cell Line, to Predict Immune-Suppressive Potency

of Human Bone-Marrow Stromal Cells (BMSCs) In Vitro: A Pilot Study, *International Journal of Molecular Sciences,* 24 (17) : 13258 (IF: 5,6)

Balázs Széky, Balázs Mayer, Miklós Gyöngy, Anna Hajdara, Szilvia Barsi, Sarolta Kárpáti, Krisztián Németh (2021) Tri-Lineage Differentiation of NTERA2 Clone D1 Cells towards Neural, Hepatic and Osteogenic Lineages In Vitro, Folia Biologica 67 : 5-6 pp. 174-182. , 9 p (IF: 0,6)

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