supported by the fact that mice bearing tumors induced by BGNhigh K-RAS G12V+ cells showed a reduced MHC class I expression, which was associated with an enhanced frequency of CD8+ and CD4+ cells in the peripheral blood.

Conclusions Our data provide evidence that (i) proteoglycan signatures are modulated by K-RAS G12V transformation, (ii) loss of proteoglycan expression is directly or indirectly involved in immune escape of K-RAS G12V overexpressing tumor cells and (iii) BGN overexpression and enhanced basal decorin expression results in altered growth properties of K-RAS G12V cells. Thus, the reduced migration rate and restoration of MHC class I surface expression by BGN or other proteoglycans are important features for their anti-tumorigenic properties in K-RAS G12-transformed tumor cells including colorectal cancers.

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Consent Not applicable

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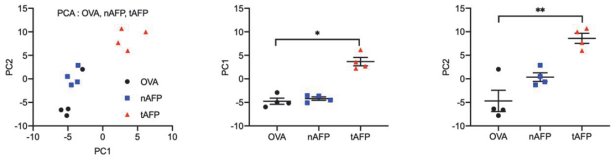
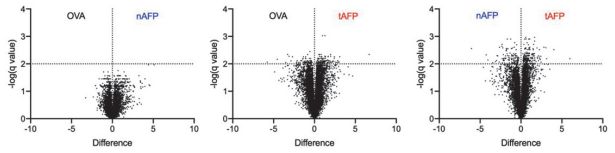


Abstract 829 Figure 1 tAFP induces a distinct gene expression profile in monocyte-derived DC's



Abstract 829 Figure 2 Identifying differentially expressed genes in OVA, nAFP, and tAFP treated DC's

829 TUMOR ALPHA-FETOPROTEIN INHIBITS CHOLESTEROL AND STEROID METABOLISM IN MONOCYTE-DERIVED DENDRITIC CELLS

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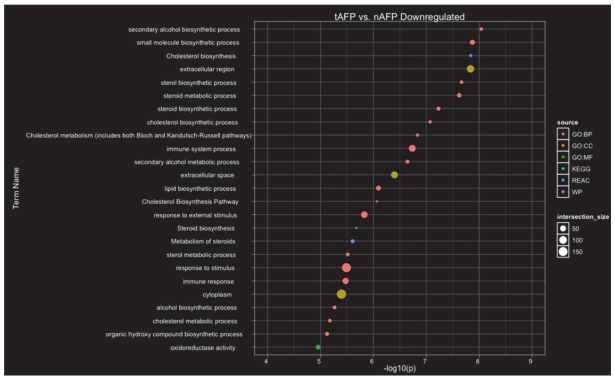
Background Hepatocellular carcinoma (HCC) is a particularly lethal malignancy in part due to the potently immune-suppressive tumor microenvironment. The weak immune response is due in part to the presence of tumor alpha-fetoprotein (tAFP), a fetal glycoprotein that is produced by a majority of HCC tumors.[1] Previously, we showed that tAFP potently inhibited the differentiation of monocytes to dendritic cells when compared to cord blood-derived normal AFP (nAFP) and ovalbumin (OVA).[2] Additionally, we demonstrated that tAFP inhibits lipid metabolism by limiting the expression of fatty acid metabolic enzymes.[3] To identify the mechanism whereby tAFP alters dendritic cell metabolism, we analyzed microarray data by a functional enrichment pathway analysis with g: Profiler.[4]

Methods Monocytes from healthy donors (n=4) were isolated with CD14 magnetic beads and differentiated for five days in the presence of IL-4 and GM-CSF with OVA, nAFP, or tAFP. After five days, we isolated RNA for microarray analysis using an Affymetrix HG-U133A array. R studio generated principal component analysis. Differentially expressed (DE) genes were identified as a 1 log fold change and had adjusted p values of

Results Principal component analysis of the gene expression data revealed that tAFP clustered separately from OVA and nAFP based on PC1 (p = 0.016) and PC2 (p = 0.009) (figure 1). In total, 688 DE genes were identified with 495 upregulated and 193 downregulated (figure 2). Downregulated DE genes between tAFP versus nAFP yielded significantly down regulated pathways including cholesterol (p = 10e-7.5), steroid (p = 10e-7.5), and lipid biosynthesis (p = 10e-6) (figure 3). Interestingly, upregulated DE genes between tAFP versus nAFP included many pathways specific to stress response to metal ions including zinc (p = 10e-10.5) and copper (p = 10e-10) (figure 4).

Conclusions In addition to validating previous data demonstrating tAFP inhibited lipid biosynthesis generally, this is the first report to our knowledge of tAFP inhibiting gene signatures associated with cholesterol and sterol synthesis specifically. Furthermore, we identified significant upregulation of



Abstract 829 Figure 3 tAFP downregulates cholesterol and steroid metabolism in DC's

Abstract 829 Figure 4 tAFP upregulates stress response to metal ions in DC's

gene pathways corresponding to the stress response genes to metal ions. Notably, functional assays are underway to confirm these gene pathway data. These findings shed new insight into how tAFP perturbs monocyte and DC metabolism and thereby limits differentiation of monocytes to immature dendritic cells. Future insights into how tAFP limits innate immunity could lead to improved immunotherapies for HCC.

Ethics Approval Samples were collected with informed consent at the University of Pittsburgh (Pitt IRB #UPCI 04-001 and UPCI 04-111).

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830 TARGETING CELLULAR SENESCENCE TO INCREASE CAR-T CELL FITNESS

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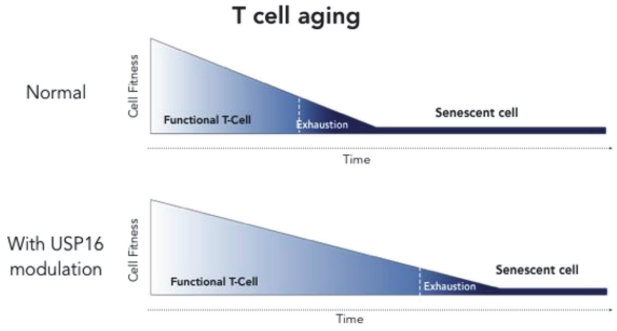
Background Immunosenescence refers to the age-associated decline of the adaptive immune system, which results in increased incidence and severity of infections, cancers, and autoimmunity. The elderly show reduced numbers of naïve T cells, skewed CD4:CD8 ratio, reduced proliferative and functional capabilities, and increased expression of senescence markers. These phenomena have strong repercussion in adoptive immunotherapy.

Notably, the ex vivo manufacturing process of CAR-T cells per se induces senescence extremely quickly; 15 days of T cell expansion age cells 30 years, as measured by telomere length, T cells differentiation and CDKN2a mRNA levels.

To circumvent this problem, we here propose the modulation of USP16, an epigenetic regulator of stem cells and senescence in multiple tissues. Downregulation of USP16 rejuvenates T cells, offering a powerful tool to dramatically improve the efficacy of CAR-T treatments.

Methods During ex vivo CAR-T cell manufacture, cells age very rapidly, strongly decreasing T cell fitness. Importantly, we observed that cellular senescence is an early event that precedes T cell exhaustion upon CD3/CD28 T cell stimulation, making it a very interesting pathway to target. In line with this hypothesis, we demonstrated that reducing cellular senescence increases CAR-T cell functions both in vitro and in vivo.

Results We identified an epigenetic regulator, USP16, whose mRNA levels increase during T cell expansion and correlate with the expression of the aging marker par excellence, CDKN2a. Genetic modulation of USP16 in CD19 and GD2 CAR-expressing T cells not only reduces senescence markers but also expands the naive (CD45RA+CD62L+) population and enhances cell self-renewal, without negative effects on T cell expansion. USP16 modulation also results in increased killing, polyfunctionality, and expansion upon in vitro stimulation with tumor cells. Notably, the delay of cellular senescence induces long-lasting cellular fitness (figure 1) as T cells are less exhausted upon multiple tumor challenges. In vivo, T cells rejuvenated by USP16 modulation, are 60% more efficient in controlling tumor growth in a mouse model of leukemia (NALM-6) and neuroblastoma (CHLA-255).



Abstract 830 Figure 1 Effect of USP16 modulation in T cell aging
The schematic shows the relation between cell functionality, exhaustion and cellular senescence in normal T cell aging (top) and when USP16 is inhibited (bottom). USP16 modulation reduces T cell aging, increasing cell functionality and delaying exhaustion and cellular senescence.

Conclusions We demonstrated that modulation of USP16 prevents cellular senescence and increases self-renewal in T cells. This approach can significantly improve CAR-T therapy in multiple diseases, including leukemias and solid tumors. Development of small molecules against USP16 could offer a viable solution to improve T cell fitness during manufacturing.

Ethics Approval The study was approved by Institutional Animal Care and Use Committees (IACUC), approval number CR-0104.

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831 E3 UBIQUITIN LIGASE CBL-B DEFICIENT CD8+ T CELLS OVERCOME TREG CELL-MEDIATED SUPPRESSION THROUGH IFN-γ AND INDUCE ROBUST ANTI-TUMOR IMMUNITY

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Background Adoptive T cell therapy (ACT) is reaching its potential in multiple malignancies. However, anti-tumor T cell responses can be attenuated by suppressive cells in the tumor microenvironment, such as CD4+FoxP3+ regulatory T (Treg) cells. Depletion of Treg cells can be technically challenging in ACT and may be associated with unwanted adverse effects. Alternatively, studies suggest that specific modifications in T cell signaling network may render T cells resistant to regulation by Treg cells. Here, we investigated the role of Casitas B- Lineage Lymphoma-b (Cbl-b), an E3 ubiquitin ligase and a negative regulator of TCR signaling pathways, in rendering CD8+ T cells resistant to the effects of Treg cells to bolster ACT.