

TUMOR-DERIVED ALPHA-FETOPROTEIN REQUIRES POLYUNSATURATED FATTY ACIDS FOR IMMUNO-METABOLIC DYSREGULATION

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Background Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer deaths worldwide.¹ The immuno-regulatory environment of the liver, coupled with tumor-specific immuno-suppressive mechanisms, has negatively impacted the development of clinically effective immunotherapies. Most HCC tumors secrete alpha-fetoprotein (AFP), which we previously demonstrated inhibited monocyte to dendritic cell (DC) differentiation and metabolism.²⁻³ These immunoregulatory effects depended upon a previously unidentified low molar mass ligand bound to tumor-derived (tAFP) but not cord-blood-derived 'normal' AFP (nAFP). To delineate the mechanism, we identified and tested fatty acids (FA) unique to tAFP necessary for immunosuppression.

Methods Fatty acids bound to samples of ovalbumin (OVA), nAFP, and tAFP (n=3 each), were quantified by mass spectrometry and gas chromatography by the UCSD Lipidomics Core. Analysis of the single-cell metabolism was measured using the SCENITH assay⁴ via spectral-flow cytometry. Bulk measurement of metabolism was measured by microarray and glucose/lactate quantification of supernatants during monocyte to DC differentiation in vitro. Lastly, several fatty acids (FAs) were co-incubated with ligand-free preparations of OVA, nAFP, and tAFP to determine which FAs contribute to limiting DC differentiation in vitro.

Results SCENITH analysis revealed a stark increase in lactate secretion and a marked switch from oxidative-phosphorylation (OXPHOS) to glycolysis in tAFP-treated DCs, which correlated with reduced co-stimulatory marker expression and increased PD-L1. g:Profiler analysis of microarray data confirmed dysregulation of FA metabolism. We identified three polyunsaturated fatty acids (PUFAs) that were enriched on tAFP by mass-spectrometry and gas chromatography. Screening of FAs on ligand-free preparations revealed two PUFAs on tAFP were uniquely able to decrease differentiation of iDC and mDCs in vitro.

Conclusions We have identified unique FA ligands of tAFP and determined specific FAs that restore its immunoregulatory activities. To our knowledge, these are the first data demonstrating a role of a novel PUFA in inhibiting DC formation and are consistent with previous reports showing arachidonic (20:4) inhibits DC formation in vitro.⁵ Furthermore, we have identified key metabolic pathways of the immuno-metabolic dysregulation of DCs in HCC. These findings identify targets for strategies to reverse the tAFP induced immuno-metabolic dysfunction in vivo could be a strategy to potentiate robust anti-tumor immunity and improve survival in HCC patients.

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Ethics Approval The Cancer Immunotherapeutics Tissue Use Committee approved samples from healthy donors at UCSF.

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