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Obesity-induced changes in the tumor microenvironment impact the response to chemotherapy and overall ovarian cancer metastatic success

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Background: The majority of women with epithelial ovarian cancer (OvCa) are diagnosed with metastatic disease, resulting in a poor 5-year survival of 31%. Obesity is a recognized epidemic that increases OvCa incidence, enhances metastatic success and reduces survival. We have previously demonstrated a link between obesity and OvCa metastatic success in a diet-induced obesity (DIO) mouse model where a significantly enhanced tumor burden was associated with a decreased M1/M2 tumor-associated macrophage (TAM) ratio (Liu Y et al. Can, Res. 2015; 75:5046-57). TAMs are the most abundant immune cell type in the ovarian tumor microenvironment and are generally categorized as M1-polarized (cytotoxic to tumor cells), or M2-polarized (growth promoting). For this study we sought to examine if a similar correlation between body mass index (BMI) and TAMs were also true for human ovarian cancer patients. Furthermore, we used the DIO mouse model to examine any potential effect of obesity on response to chemotherapy.

Methods: We analyzed high grade serous ovarian tumors from 6 normal weight patients (BMI 20-25), and 8 obese patients (BMI ≥30) for an in-depth analysis of immune cells using MultiOmyx™, an immunofluorescence (IF) multiplexing assay. After multiplexing FFPE sections with a custom panel of 13 immuno-oncology biomarkers (see table 1), images were analyzed by applying the deep-learning based cell classification platform NeoLYTX.
For the analysis of response to chemotherapy we used a DIO mouse model in which mice were fed a low-fat diet (LFD, 10% fat, Research Diets #D12450J) *vs* high-fat diet (HFD, 45% fat, Research Diets #D12451) and then injected with RFP-tagged ID8-Trp53$^{-/-}$ cells (10^6) to establish tumor burden. Mice bearing equivalent tumor burden were treated with weight-adjusted standard of care chemotherapy [paclitaxel (6mg/kg) and carboplatin (15mg/kg), designated **PC**]. After sacrifice, remaining tumor burden was evaluated by quantitative fluorescence imaging.

Results: We found that the mean M1/M2 ratio in obese OvCa patients was nearly a third (ratio=0.11) compared to patients who were normal weight (ratio=0.34), consistent with our previously published mouse data. Quantitation of residual tumor burden in the mouse model showed significantly reduced efficacy of chemotherapy (i.e., greater remaining tumor burden) in HFD mice (n=11/cohort). When these tumors were evaluated by IHC for M1/M2 markers iNOS and CD206 we observed a decrease in the M1/M2 ratio in HFD tumors relative to LFD controls, corresponding to the results from our OvCa patient analysis.

Conclusions: Our observations suggest that the negative impact of obesity on OvCa survival may be due in part to a decreased M1/M2 TAM ratio and reduced response to chemotherapy. These data will be key to a more detailed understanding of the contribution of host obesity to ovarian tumor progression.

MultiOmyx™ panels and co-expressions

13-Marker Panel	
CD3	CD68
CD4	HLA-DR
CD8	CD163
FoxP3	E-cad
CD45RO	Vimentin
PD-1	PanCK
PD-L1	

Tables 1+2.

Co-expression	Phenotypes
CD3+CD4+	T helper
CD3+CD4+FoxP3+	T regulator
CD3+CD8+	T cytotoxic
CD3+CD45RO+	Memory T cell
CD3+PD1+	Exhausted T cell
CD68+HLADR+	M1 TAM
CD68+CD163+	M2 TAM
CD68+PDL1+	PD-L1+ TAM
PanCK+PDL1+	Tumor+ PD-L1

9-Marker Murine Panel
CD3
CD4
CD8
FoxP3
CD44
CD68
PD-L1
Vimentin
PanCK

Co-expression	Phenotypes
CD3+CD4+	T helper
CD3+CD4+FoxP3+	T regulator
CD3+CD8+	T cytotoxic
CD68+CD44+	TAM CD44+
CD68+PDL1+	TAM PD-L1+
CD68+vimentin+	TAM vimentin+
PanCK+PDL1+	Tumor cell PD-L1+
PanCK+CD44+	Tumor cell CD44+

Tables 3+4.

Tables 1-4. For MultiOmyx multiplexing two conjugated fluorescent antibodies are applied per round, followed by image acquisition of the stained slides. The dye is erased, enabling a subsequent round of staining with another pair of fluorescent antibodies. **Table 1)** 13-marker protein panel composition. **Table 2)** Phenotyping of human immune cells. **Table 3)** 9-marker murine protein panel composition. **Table 4)** Phenotyping of murine immune cells. TAM: tumor-associated macrophage.

Analysis and multiplex images of OvCa patient tumors

Patient #	BMI	Height (cm)	Weight (cm)	Age	Histological Type	Stage (final)	Grade
1	20.3	157.5	50.3	79	ADENOCARCINOMA, NOS		
2	20.5	162.3	54	56	PAP. SEROUS ADENOCARCINOMA	IIIC	
3	21.3	165.1	58.1	65	PAPILLARY SEROUS CARCINOMA		
4	21.4	170	61.9	62	SEROUS CARCINOMA	IIIC (FIGO)	High Grade
5	21.7	162	57	76	SEROUS ADENOCARCINOMA	IIIC (FIGO)	High Grade
6	22.4	150	50.3	62	PAP. SEROUS & ENDOMETRIOID AC		High Grade
7	32.8	160	84	55	SEROUS CARCINOMA	IIIC (FIGO)	High-grade
8	33.7	161.1	87.5	70	SEROUS ADENOCARCINOMA	IIIC	High Grade
9	33.7	156.9	83	78	PAPILLARY SEROUS CARCINOMA		
10	35.5	160	90.9	53	SEROUS CARCINOMA		High Grade
11	36.3	160	92.8	63	SEROUS CARCINOMA	IIIC (FIGO)	High Grade
12	38.4	161.3	99.9	59	PAPILLARY SEROUS CARCINOMA		
13	38.8	169.8	112	54	PAPILLARY SEROUS CARCINOMA		3/3
14	42.2	170	122	52	SEROUS ADENOCARCINOMA		High-grade

Figure 1. Clinical data from patients evaluated in this study. The cohort of normal weight patients is comprised of patients 1-6 (BMI 20-30), while the cohort of obese patients is comprised of patients 7-14 (BMI > 30).

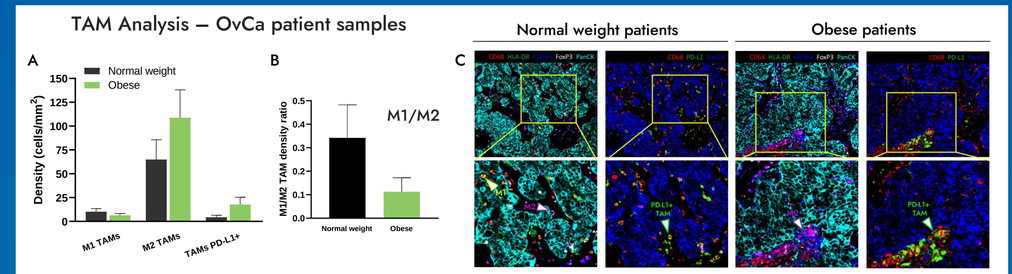

Figure 2. Analysis of TAMs in OvCa patient tumors. Quantification results of the density of M1 TAMs, M2 TAMs, and TAMs positive for PD-L1 were generated by applying the proprietary deep-learning based cell classification platform NeoLYTX to MultiOmyx multiplexed IF images. **A+B.** We observed a decrease in M1 TAM density and an increase in M2 TAM density in the obese OvCa population, leading to an M1/M2 ratio of 0.11 in obese patients vs. a 0.34 ratio in normal weight patients. **C+D.** Representative color overlay images of tumors from normal weight and obese OvCa patients. Arrows indicate examples of M1 TAMs (yellow), M2 TAMs (magenta), or PD-L1 positive TAMs (green). Conclusion: *A decrease in the M1/M2 ratio is observed in tumors from obese OvCa patients relative to normal weight patients.*

Analysis and multiplex images of murine tumors

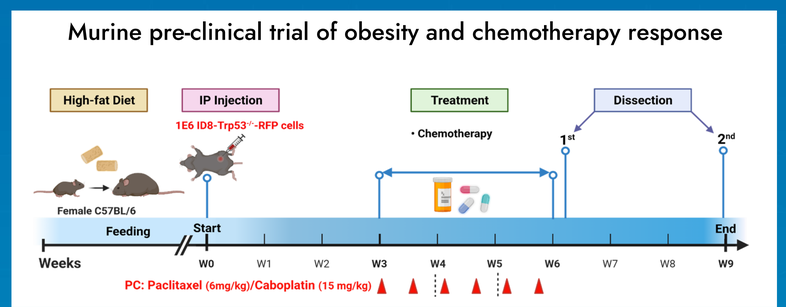

Figure 3. Study overview. For the analysis of response to chemotherapy we used a diet-induced obesity (DIO) mouse model in which mice were fed a low-fat diet (LFD, 10% fat, Research Diets #D12450J) *vs* high-fat diet (HFD, 45% fat, Research Diets #D12451) and then injected with RFP-tagged ID8-Trp53$^{-/-}$ cells (10^6) to establish tumor burden. Mice bearing equivalent tumor burden were treated with weight-adjusted standard of care chemotherapy [paclitaxel (6mg/kg) and carboplatin (15mg/kg).

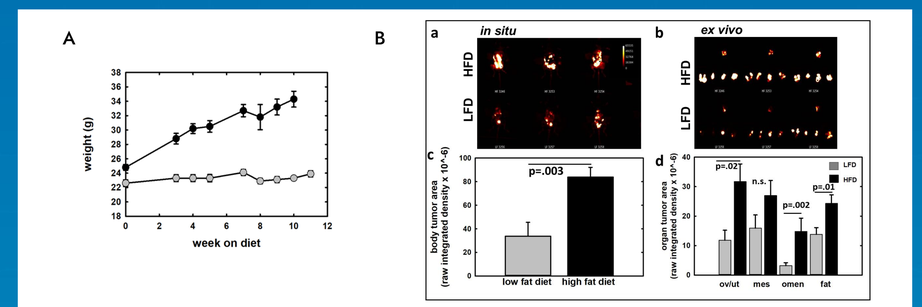

Figure 4. Poor response to chemotherapy in high fat diet mice. A. C57/Bl6 mice (n=11/cohort) were fed a control low fat diet (LFD, 10% fat, Research Diets #D12450J) or high fat diet (HFD, 45% fat, Research Diets #D12451) until an average 10g difference in weight was achieved ($p<0.001$), then injected i.p. with RFP-tagged ID8-Trp53$^{-/-}$ cells (10^6). **B.** Tumor-bearing mice were monitored by longitudinal *in vivo* imaging for development of equivalent tumor burden, then treated with 9 cycles of weight-adjusted chemotherapy [paclitaxel (6mg/kg) and carboplatin (15mg/kg), designated PC]. **(Ba,c)** Mice were sacrificed and remaining tumor burden was imaged *in situ* following a midline dissection and quantified. **(Bb,d)** Peritoneal organs were removed, imaged *ex vivo*, and remaining tumor burden quantified. Conclusion: *HFD mice have significantly greater remaining tumor burden, indicative of a poor response to standard-of-care chemotherapy .*

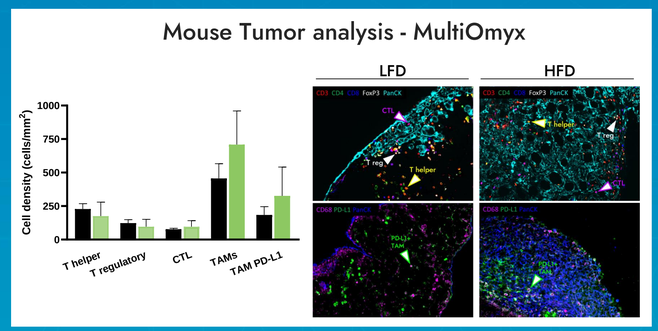

Figure 5. Analysis of immune cells in omental tumors. Quantification results of immune cells were generated as described for figure 1. **A**. We observed an non-significant increase in the density of total TAMs in the HFD group. **C.** Representative color overlay images of tumors from LFD and HFD cohorts. Arrows indicate examples of indicated immune cell. Conclusion: *An increase in total TAM density is observed in HFD tumors relative to LFD controls.*

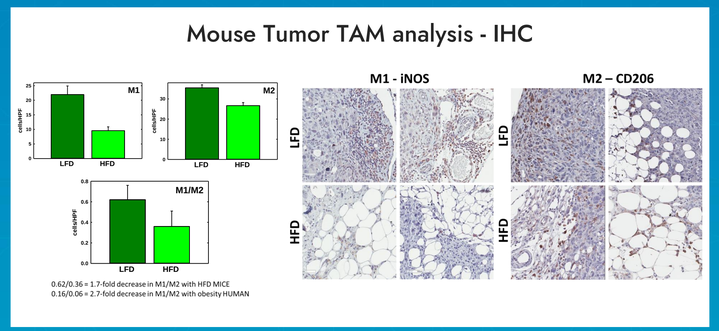

Figure 6. Analysis of TAMs in omental tumors. Omental tumors from the LFD and HFD cohorts shown in Fig. 4 were sectioned and stained for M1 macrophages (rabbit anti-iNOS, 1:1000. Abcam, #ab15323) or M2 macrophages (rabbit anti-CD206, 1:6400, Abcam #ab64693). Positively stained macrophages were enumerated in 10 fields/tumor. Conclusion: *A decrease in the M1/M2 ratio is observed in HFD tumors relative to LFD controls.*

Key Takeaways

- We observe a decreased M1/M2 TAM ratio in obese OvCa patients, as well as in tumor-bearing mice on a HFD (High-fat Diet).
- The HFD blunts response to chemotherapy in our murine model, suggesting a negative impact of obesity on OvCa survival.

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