Title: Lipid Droplets drive Genetic Changes but impede Tumoroid Growth without affecting Macrophage-mediated elimination

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Introduction:

Obesity is a major risk factor for 13 cancer types and 40% of all cancers, according to current CDC analyses. Tumor heterogeneity results in resistance to cancer treatments (Marusyk et al., 2009), understanding what drives such evolution is important to developing more effective therapeutics. Lipid droplet (LD) accumulation in tumors induces morphological changes and increases malignant behavior in cancer cells (Cruz et al., 2020). LDs were discovered to be rigid enough to deform the cytoskeleton and nucleus, even causing nuclear envelope rupture and increased DNA damage (Ivanovska et al., 2023), which could increase genetic instability. The impact of LDs on cancer cells must thus be considered when characterizing cancers.

Because LDs delay the cell cycle in 2D cultures (Ivanovska et al., 2023), they could also slow tumoroid growth in 3D. This could create vulnerability by enhancing the ability of immune cells, including macrophages, to efficiently attack cancer cells. Macrophage immunotherapy is indeed emerging as a possible therapy for cancer, due to their phagocytic behavior and the possibility of initiating acquired immunity.

We tested the impact of lipids on B16 mouse melanoma cells in 2D and 3D tumoroid culture, quantifying genetic instability and tumor growth. To better understand how lipids influence immune interactions, we co-cultured macrophages with lipid-treated tumoroids (opsonized and non-opsonized) to observe differences in phagocytic behavior between treated and untreated co-cultures. Engineered macrophages with knockout of SIRP-α (the macrophage checkpoint receptor protein for CD47) were used to help drive phagocytosis.

Methodology:

To evaluate the genomic instability of B16 mouse melanoma cells due to lipid stress in 2D culture, cells were seeded in six-well plates incubated in cell media containing 1mM oleic acid (OA) for 3 days with the general lipid stain Bodipy. The lipid-stressed cells were then fixed, DNA stained, and imaged using fluorescence microscopy. Using a chromosome reporter system (ChReporter), where genetically engineered cells express GFP alongside the Lamin-B1 gene on chromosome 18, genetic changes were identified through imaging. Using ImageJ, the stressed cells were also analyzed for gain/loss colonies of GFP signal, micronuclei/enlarged nuclei formation, cell division rate, and lipid uptake.

To assess the impact of LD stress in 3D culture, mCherry-expressing B16 cells were similarly stressed as in 2D. After confirmation of lipid loading, they were detached and transferred to a 96-well U-bottom plate with anti-adhesive coating, enabling tumoroid formation 24 hours later. SIRP-α knockout conditionally immortalized macrophages (SKO) and TA99 monoclonal antibodies (to opsonize melanoma cells) were added to some tumoroids for co-culture.

In parallel, other mCherry-expressing B16 cells were seeded at various cell densities to generate tumoroids overnight at 37 °C. One group received 1mM OA treatment for 72 hours, and a control group was also set aside for comparison. All tumoroids were imaged every 24 hours using fluorescence microscopy and analyzed for growth using ImageJ.

Results:

LD formation in B16 cells in 2D cultures increased after 72 hours of incubation with OA, indicating that lipid loading was successful. Noting that B16 cells double every 12-15 hours, LDs decreased the number of cell divisions in B16s cultured in 2D, while increasing the percentage of cells with micronuclei. B16s with LDs also showed 0.4% ChReporter loss, while no ChReporter loss was observed in B16s that did not receive LDs. Our results support the idea that LDs stress these mouse melanoma cells, leading to fewer divisions but increased chromosome segregation errors per division, as seen by the increased micronuclei and ChReporter signal loss. Similar results were observed with human osteosarcoma U20S cells.

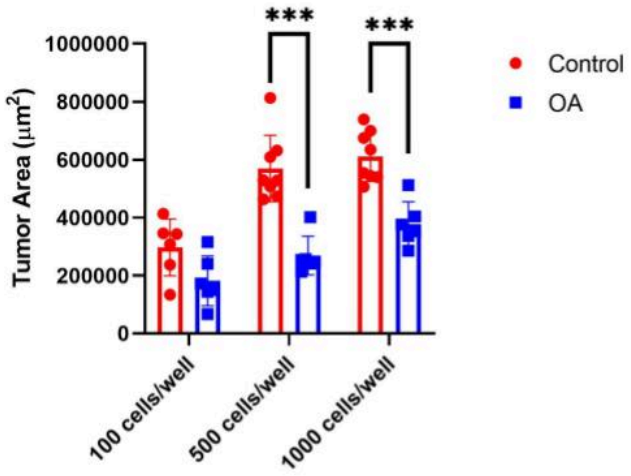

Figure 1. Differences in tumoroid area between tumoroids that received OA and those that did not.

Tumoroids in 3D of B16 melanoma cells with OA, added to form LDs, show 40-50% ($p < 0.01$) reduced tumoroid areas on day 5 relative to untreated tumoroids regardless of cell densities (Figure 1). This again suggests that lipids inhibit cancer cells from proliferating, which is consistent with our 2D results for B16 mouse melanoma cells. Furthermore, tumoroids derived from lipid-stressed cells do not grow when macrophages are added with an antibody that drives phagocytosis. Lipid stress has no significant effect on tumoroid area on day 5 when co-cultured with macrophages. This suggests that oleic acid has minimal impact on macrophage phagocytosis under maximal conditions for engulfment.

Overall, our results indicate that lipids delay cell division in B16 cells in 2D and 3D while also increasing genomic instability, indicating that they contribute to tumor heterogeneity and cancer evolution even if they impede growth. Furthermore, the macrophage phagocytic behavior

is unaffected by lipid stress, which is promising for a therapy that can initiate acquired immune responses against the evolving tumor as driven by LDs.

Sources:

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