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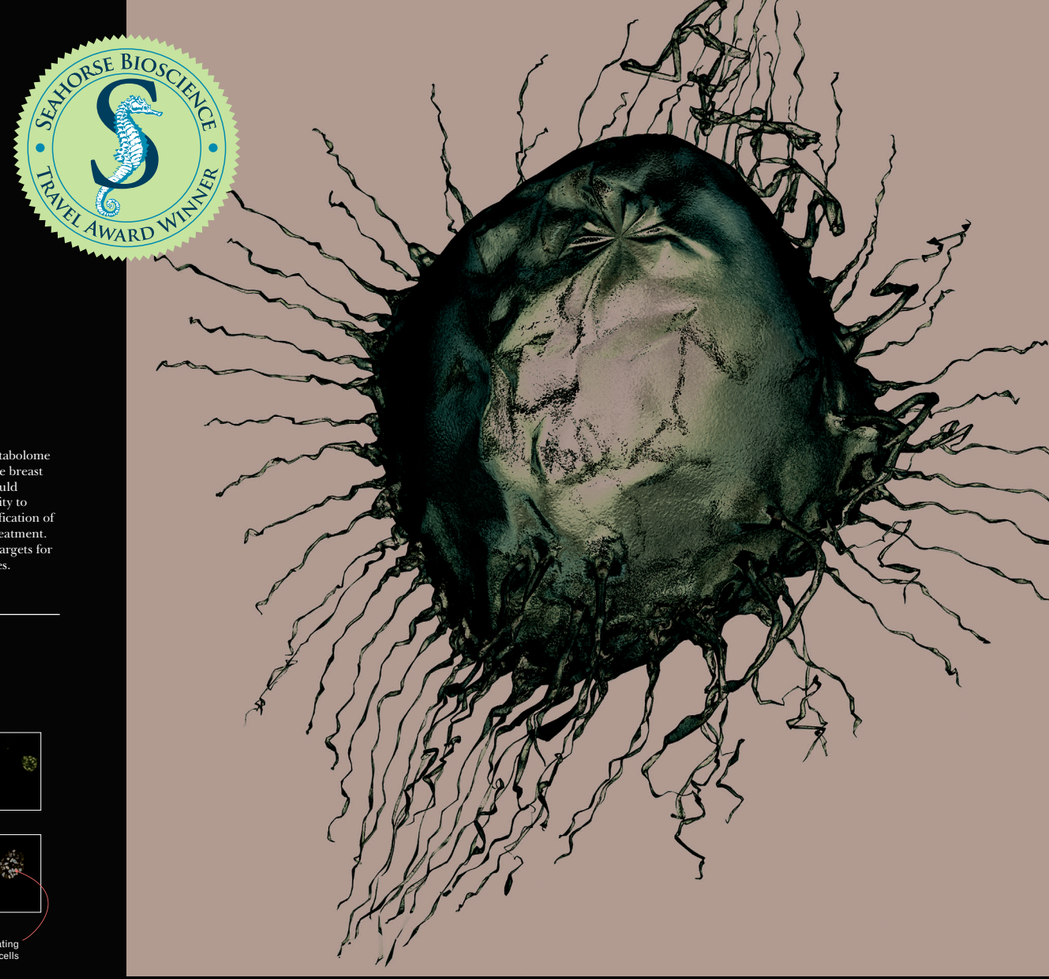
Breast cancer, statins and 3D cell culture



The need for new treatments

Breast cancer affects 11% of women in North America *(1)*. Currently, half of treated women relapse. Treatment depends on type of tumor, with triple-negative tumors having the fewest therapeutic options. There is a need for new treatments tailored to those most likely to respond. Statins are a possibility.

Statins: Anti-cholesterol drugs

Statins are used to lower plasma LDL cholesterol levels *(2)*. They inhibit HMGCR, the rate-limiting enzyme of the mevalonate pathway, reducing cholesterol synthesis. This activates SREBP transcription factors, increasing LDL-receptor levels and the uptake of LDL cholesterol from plasma.

Why are some tumors statin-sensitive?

Statins also deplete other mevalonate-pathway intermediates, potentially explaining their apoptotic action in *in vivo* and *in vitro* cancer models. Responses vary depending on the type of statin and tumor. Uncovering determinants of statin sensitivity is essential for better tailoring of treatment.

3D cell culture: A better model?

3D culture could be more representative of a breast tumor than traditional 2D methods. 3D gene expression and morphologies have been extensively characterized. Whether treatments have similar effects in 3D culture to those in 2D is not yet known. We will characterize responses to fluvastatin in both 2D and 3D.

Effect of statins on metabolism

Tumor cells often have altered metabolism, so targeting altered metabolic pathways in cancer is an attractive proposition. Statins can change mitochondrial activity—and potentially other metabolic pathways—in normal cells. We aim to uncover whether this also occurs in cancer cells.

The future

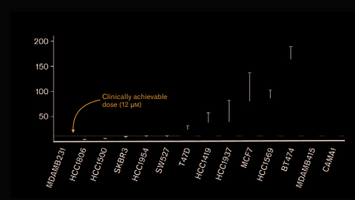
We aim to characterize the metabolome of both sensitive and insensitive breast cancer cell lines in 3D. This could uncover a signature of sensitivity to fluvastatin, allowing the identification of patients likely to respond to treatment. Differences could also hint at targets for potential combination therapies.

TWO DIMENSIONS

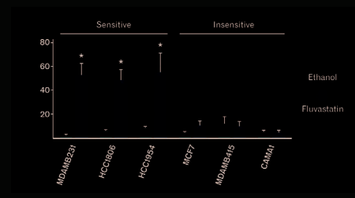
Breast cancer cell lines show a range of sensitivities to fluvastatin

Our lab has already shown that sensitivity to statins varies among multiple myeloma cell lines *(3)*. We have now expanded our research into breast cancer cell lines. Fluvastatin has a range of anti-proliferative effects in the cell lines: a strong effect in MDAMB231 cells and no effect in CAMA1 cells (Fig. 1). A selection of fluvastatin-treated sensitive cell lines show increased apoptosis, which is not seen in a representative selection of insensitive cell lines (Fig. 2).

1 FLUVASTATIN REDUCES VIABILITY IN SOME CELL LINES



2 FLUVASTATIN INDUCES APOPTOSIS IN SENSITIVE CELL LINES



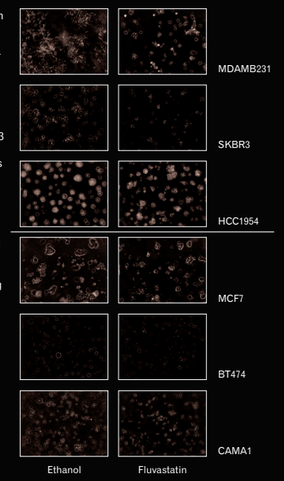
1 Cells were treated for 72 h with a range of concentrations of fluvastatin. MTT was added at the end of the treatment. MTT absorbance was measured and compared to the untreated control. Values shown are the concentrations of fluvastatin required to reduce viability by half. (n = 3; mean ± SD)

2 Cells were treated for 72 h with 10 μM fluvastatin, harvested and fixed with 70% ethanol. The cells were stained with TUNEL, and positive cells detected using flow cytometry. Apoptotic cells stained positive, and are shown as a percentage of total cells. (n = 3; mean ± SD)

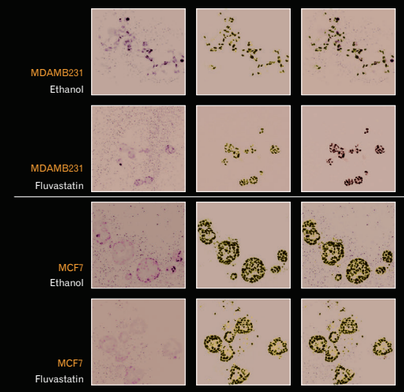
SENSITIVITY CAN BE SCORED IN 3D BREAST CANCER CELL MODELS

3 MORPHOLOGICAL CHANGES OF 3D CELL CULTURES INDUCED BY FLUVASTATIN

Cells were grown on Matrigel for 4 days, then treated with 10 μM fluvastatin for 72 h. The order of cells sensitive to fluvastatin in 2D is kept in 3D (judged qualitatively). MDAMB231, SKBR3 and HCC1954 are sensitive, the others are not. 3D breast-cancer cell lines show a variety of morphologies, which complicates analysis. We intend to use IHC as a more quantitative approach to scoring sensitivity. Images are representative of 3 independent experiments.



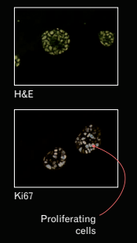
4 IMMUNOFLUORESCENCE OF FLUVASTATIN-TREATED CELLS IN 3D



Statin-sensitive MDAMB231 and insensitive MCF7 cells were grown on Matrigel for 4 days, then treated with 10 μM fluvastatin for 48 h. Structures were fixed in formalin and stained with a Ki67 antibody. Throughput can be increased using immunohistochemistry.

5 IHC OF HCC1954 CELLS IN 3D

HCC1954 cells were grown on Matrigel for 6 days. 3D structures were fixed in formalin, embedded in paraffin and stained with either H&E or Ki67. We have optimized this technique and will now use it for high-throughput quantification of the effects of fluvastatin on cell lines grown in 3D.



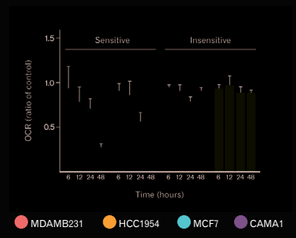
We aim to characterize the metabolome of sensitive and insensitive breast cancer cell lines in 3D

METABOLISM

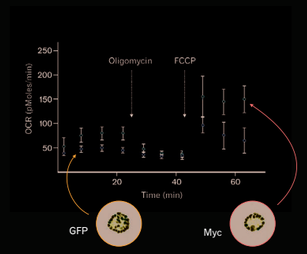
Can fluvastatin affect mitochondria and metabolism?

Many cancer cells have an altered metabolism compared to normal cells *(4)*. Proliferating cancer cells require increased production of ATP and biosynthetic molecules. Drugs targeting tumor metabolism are in the clinic (e.g. 5-fluorouracil) or in clinical trials (e.g. arginine deiminase). Statins can affect mitochondrial activity in normal cells *(5)*. We now show that fluvastatin reduces mitochondrial activity in a selection of sensitive cells (Fig. 6). We have started to optimize measuring mitochondrial activity in 3D culture, using MCF10A cells over-expressing Myc to induce an up-regulation in mitochondrial activity (Fig. 7).

6 OXYGEN CONSUMPTION IS IMPAIRED IN SENSITIVE CELLS



7 OXYGEN CONSUMPTION CAN BE MEASURED IN 3D CELL CULTURE



6 Cells were treated with 10 μM fluvastatin for the time indicated. Oxygen consumption rate (OCR) was measured using a Seahorse XF96 bioanalyzer. Values are normalized to ethanol controls for each timepoint. SEM bars are also shown. (n = 2–5)

7 MCF10A cells over-expressing Myc or GFP control were grown on Matrigel for 8 days. At 8 days, the acini lumens begin to hollow via apoptosis. Myc over-expression causes more transformed acini and increased OCRs. The XF96 was used for measurements and OCRs were normalized to DNA content. Values are representative of 3 independent repeats.

KEY POINTS

1. Fluvastatin has potential as a therapeutic agent against breast cancer.
2. Immunohistochemistry is a high-throughput technique to score drug sensitivity in 3D cell culture.
3. Fluvastatin reduces mitochondrial activity in sensitive breast cancer cell lines.

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IMAGE ARTIST'S IMPRESSION OF A CANCER CELL. © MICHAEL TAYLOR/SHUTTERSTOCK.