Breast cancer, statins and 3D cell culture

The need for new treatments for breast cancer is a key focus of the National Cancer Institute (NCI). Currently, half of all patients are diagnosed with advanced breast cancer, with type of tumor, histology and hormone receptor status affecting treatment options. There is a need for new treatments tailored to breast most likely to respond. Statins are a possibility.

**Sensitivities to fluvastatin can be scored in 3D breast cancer cell models**

One limitation already shown that sensitivity to statins cannot be assessed using 2D assays, as many human breast cancer cell lines are insensitive to statins. A range of apoptosis-inducing drugs are used in 2D assays, but many are not effective in 3D models, such as the breast cancer cell line MCF7. The future may lie in 3D culture, a better model for cancer. New methods for 3D cell culture have been developed, such as Matrigel and collagen, which can be used to grow breast cancer cells in a 3D environment. These models can be used to study the effects of fluvastatin on cancer cell metabolism.

**Can fluvastatin affect mitochondria and metabolism?**

Many cancer cells have an altered metabolism compared to normal cells. Proton-driven cancer cells have increased production of ATP and increased mitochondrial activity. Drugs targeting cancer metabolism, such as statins, could be effective in 3D culture. Statins are known to reduce mitochondrial activity in normal breast cells as well as MCF7 cells. They are a possible therapeutic option for breast cancer. This could change the metabolic state of breast cancer cells and affect their sensitivity to statins. We aim to uncover a signature of sensitivity to statins in breast cancer cell lines.

**Oxygen consumption can be measured in 3D cell culture**

Oxygen consumption (OCR) is a key indicator of cell metabolism. OCR is measured using the Seahorse XFe96, a real-time metabolic screening platform. OCR can be measured in 3D cell culture, allowing for the study of cell metabolism in a 3D environment. OCRs were measured in Matrigel for 3 days, then treated with 10 µM fluvastatin for 48 h. Increased OCRs were seen in cells treated with fluvastatin, indicating increased mitochondrial activity.

**Cell viability can be measured in 3D cell culture**

Cell viability can be measured using TUNEL staining. TUNEL staining is used to detect DNA fragmentation, which is a hallmark of apoptosis. TUNEL staining was used to measure cell viability in 3D cell culture. Cells were treated with 10 µM fluvastatin for 24 h, then stained with TUNEL. TUNEL-positive cells were counted and normalized to DNA content. The results showed that fluvastatin reduced cell viability in 3D cell culture.

**Apoptosis can be measured in 3D cell culture**

Apoptosis can be measured using Annexin V-PE staining. Annexin V-PE staining is used to detect early stage apoptosis. Annexin V-PE staining was used to measure apoptosis in 3D cell culture. Cells were treated with 10 µM fluvastatin for 24 h, then stained with Annexin V-PE. Annexin V-PE-positive cells were counted and normalized to DNA content. The results showed that fluvastatin increased apoptosis in 3D cell culture.

**Mitochondrial morphology can be measured in 3D cell culture**

Mitochondrial morphology can be measured using MitoTracker Green staining. MitoTracker Green staining is used to detect mitochondrial morphology. MitoTracker Green staining was used to measure mitochondrial morphology in 3D cell culture. Cells were treated with 10 µM fluvastatin for 24 h, then stained with MitoTracker Green. MitoTracker Green-positive cells were counted and normalized to DNA content. The results showed that fluvastatin reduced mitochondrial morphology in 3D cell culture.