THE AMENDED CELL SIGNALLING OF *IN VIVO*-MIMETIC 3D STRUCTURES

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INTRODUCTION

We have developed a 3D model which mimics *in vivo* functionality

Using a clinostat we grow the hepatocellular cell line (HepG2/C3A) into functionally mature liver-mimetic spheroids. They are large (~450µm radius) and can be maintained for >300 days.

They are more predictive of *in vivo* toxicology than primary human hepatocytes. Cell lines grown in 2D cultures are essentially not predictive.

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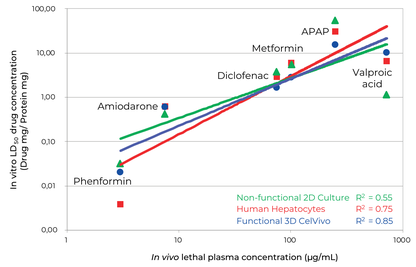

Treatment of 21 day old C3A spheroids with APAP

This clinostat model can also be used for repeated dose testing. A single high doses of APAP (20 mg / mg cellular protein) kill the spheroids (acutely lethal).
Half of that dose can kill the spheroids if given repetitively (chronically lethal).
Half the dose again produces a response after which the spheroids recover. This can be repeated many times. Lower doses again produce the same response and recovery result.

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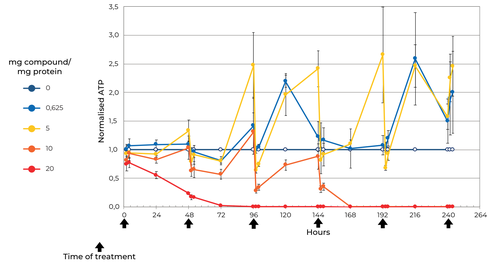

The factors that drive recovery of the physiological phenotype are hypoxia and nutrient shortage

The 3D structure results in an oxygen gradient into the spheroid (as is seen in tissues *in vivo*).

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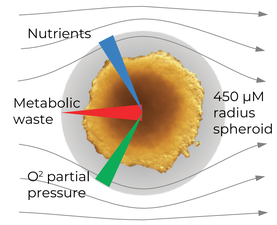

The spheroids rapidly consume all the glucose in the media. This means that they quickly become nutrient depleted.

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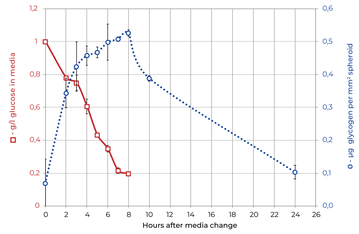

In order to determine why cells grown in 2D and 3D are so different, we analysed cells grown in 2D and 3D by mass spectrometry.

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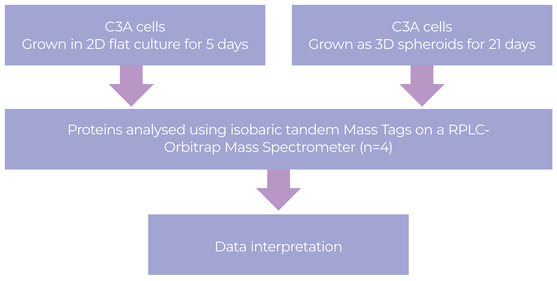

RESULTS

Hypoxia and nutrient shortage

Hypoxia and nutrient shortage appear to be the primary driver of changes in various signalling pathways leading to alterations of the cell structure and a slight decrease in oxidative phosphorylation.

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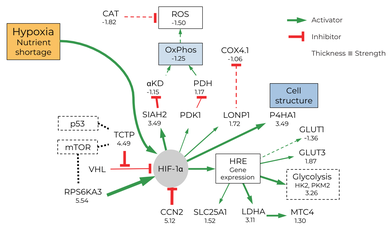

Hypoxia and nutrient shortage also affect Myc

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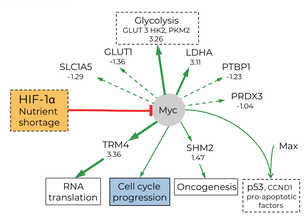

Myc affects the p53 pathway leading to changes in DNA organisation and transcription

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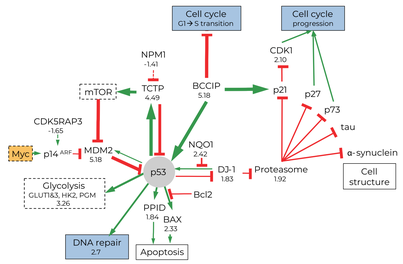

P53 and TCTP interact with the mTOR pathway increasing anabolic processes

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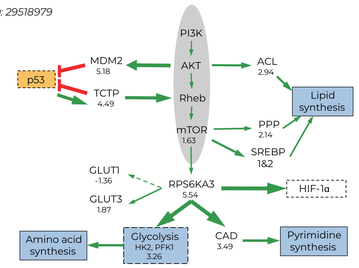

Significant changes in cell structure

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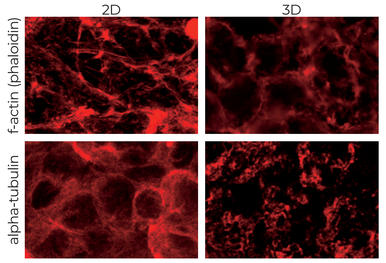

The amount of actin is unchanged but its organisation is.
Filamen A and B, profilin, cofilin, destrin, HSPH1 (↑x2)

Microtubular amount, organization and transport (in both directions) is strongly increased
Tubulin (↑x7)
Dynein and kinesin (↑x3)

Cell cycle progression slows significantly

The growth rate of cells in the 3D clinostat spheroids decreases from a doubling time of one day to 70 days after 42 days. For comparison, human liver hepatocytes are replaced every 200 – 300 days.

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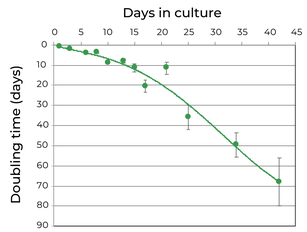

DNA repair is increased despite that there is less DNA per cell

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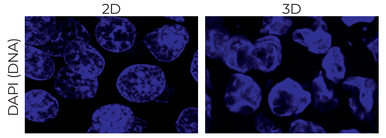

Most cells are in G_0.
Histone and proto-oncogene switching occurs.
Histone modification and clipping changes.
Changes in DNA organization.
Methyltransferase (↑x3)
DNA repair enzymes (↑x3)
DNA amount (↓x3)

Changes in signalling pathways drive metabolic reprograming from oxidative phosphorylation towards aerobic glycolysis

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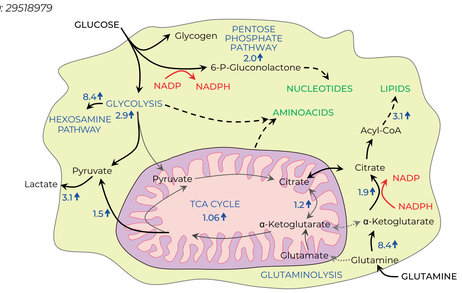
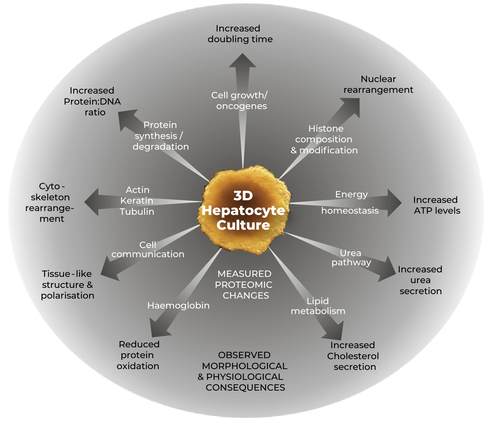


CONCLUSION

For 3D spheroids to be able to mimic human tissues, virtually every cellular process needs to be changed



The importance of various actors in signalling pathways in vivo is clearly very different to that seen in vitro in 2D cultures.

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