HORIZON DISCOVERY

Application Note

A multicellular tumor model: Applications for evaluating drugs and signaling pathways in a 3D system

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Introduction

Multicellular tumor spheroids offer many advantages over traditional two dimensional (2D) cell culture systems. Amongst these is the ability to gain insights into therapeutic problems associated with metabolic and oxygen gradients commonly found in tumors and also to directly address cell–cell and cell–matrix interactions that may influence chemoresistance mechanisms.

In this study, a multicellular spheroid model comprised of the colon carcinoma cell line HCT116 has been evaluated in order to determine the extent of the hypoxic region that is achieved. It has also been used to profile the cellular response of the bioreductive compound tirapazamine under conditions that more closely mimic the *in vivo* situation.

By screening compounds in an assay format that more accurately reflects the tumor microenvironment, compound behavior can be determined in a more clinically relevant setting at an earlier stage of the drug discovery process. Ultimately this may reduce late-stage attrition, with an associated reduction in overall timelines and costs.

Materials and Methods

For spheroid formation, HCT116 cells were seeded in ultra-low adherent 96-well plates and allowed to aggregate and form spheroids for 5 days.

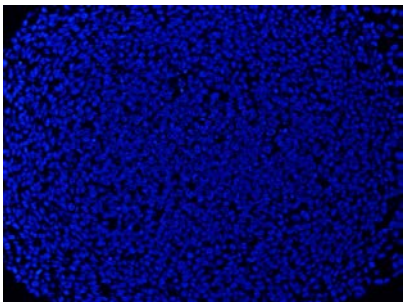
For immunohistochemical analysis of hypoxia, spheroids were treated with pimonidazole for 2h before fixation. Pooled spheroids were embedded and sectioned, before staining with H & E and with Hoechst 33258 (for cell nuclei). Hypoxia was imaged using antibody 4.3.11.3 (Hypoxyprobe-1 kit).

Tirapazamine drug treatments were performed for 4h before spheroids were dissociated and seeded into 24-well plates. Cells were then allowed to grow out for 72h before viability was measured using alamar blue.

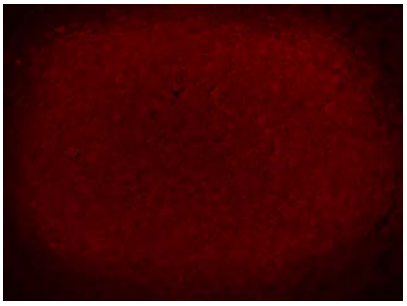
Results and Discussion

It was confirmed that HCT116 spheroids contained regions of hypoxia by performing immunochemical analysis, using the hypoxic marker pimonidazole. Detection of pimonidazole adducts, indicative of hypoxia showed that the spheroids contain a hypoxic core. The presence of normoxic cells around the periphery of the spheroid surrounding this hypoxic core is clearly shown by the merged image for cell nuclei and pimonidazole.

(A) Cell nuclei (Hoechst 33258)



(B) Pimonidazole (Hypoxia)



(C) Pimonidazole (Hypoxia)

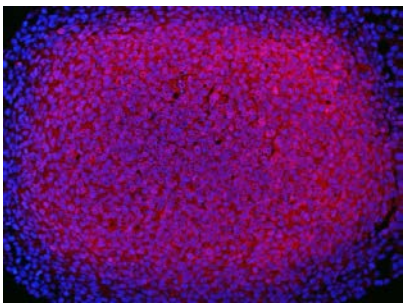


Figure 1. HCT116 spheroids comprise a normoxic outer shell, surrounding a hypoxic core.

Haematoxylin and eosin (H & E) staining demonstrated good structural integrity of the spheroids with central cells appearing necrotic.

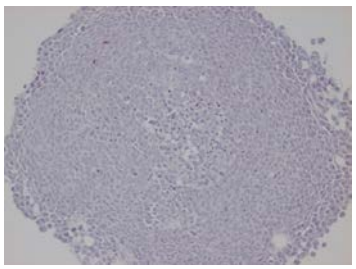


Figure 2. HCT116 3D spheroids are uniform in structure and size and contain a necrotic centre.

Tirapazamine is an aromatic heterocycle di-N-oxide that is reduced under low oxygen conditions to its active form. This produces a free radical which leads to DNA damage, and can result in cell death.

The anti-proliferative effects of tirapazamine on HCT116 cells grown in 3D were therefore investigated in order to determine whether cancer cell spheroids can recapitulate the hypoxic microenvironment typically found in tumors *in vivo*. Tirapazamine demonstrated clear anti-proliferative effects in the spheroid model, suggesting that the spheroids generated contain regions of hypoxia that are sufficient to activate a bioreductive compound.

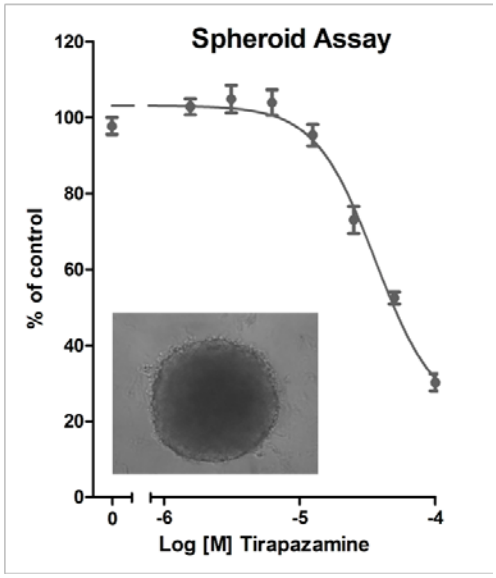


Figure 3. Tirapazamine demonstrates activity in a 3D spheroid model.

The anti-proliferative effects seen following spheroid exposure to tirapazamine were similar to those seen when 2D cultures were exposed to tirapazamine under low oxygen conditions. As can be seen in Figure 4, tirapazamine anti-proliferative activity is inversely correlated with oxygen level. Maximal anti-proliferative activity was demonstrated under anoxic (no oxygen) conditions, while intermediate anti-proliferative effects are seen under hypoxic (1% oxygen) conditions.

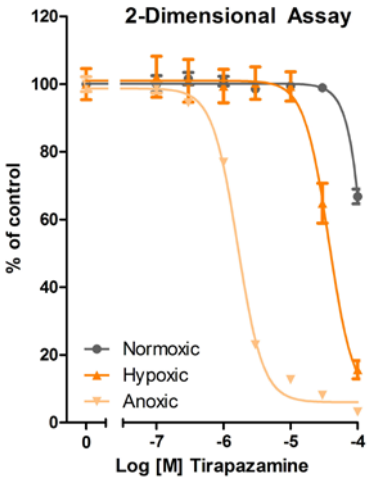


Figure 4. In a 2D cell culture assay tirapazamine requires exposure to low oxygen conditions for activation and to exert anti-proliferative effects.

Conclusion

The HCT116 multicellular tumor spheroid model can be used to demonstrate activation of bioreductive compounds. Comprised of normoxic and hypoxic cells, spheroids more closely mimic the tumor microenvironment than conventional 2D culture systems. This model may therefore be useful for investigating signaling pathways or pharmacological agents in a more tumor relevant system.

While 2D systems cannot fully mimic the tumor microenvironment, they can be useful for studies where single, well defined oxygen levels are required. Sophisticated equipment is available at Horizon Discovery that permits evaluation of either complete anoxia or precise oxygen levels ranging from 0.1% oxygen to normoxia, facilitating compound screening under a range of hypoxic conditions.

Horizon Support

Horizon supplies genetically-defined cell lines, custom cell line generation, *in vivo* models, reporter gene assay kits, molecular reference standards and assay development and screening services to organizations engaged in academic research; drug discovery and development; clinical diagnostics; and biopharmaceutical process optimization. Please contact us to learn more about how Horizon can support your work.

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