Abstract 5034

Time-lapse analysis of drug response and invasive capacity using patient-derived and CRC microfluidic 3D cancer models under oxygen controlled conditions

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INTRODUCTION

The development of relevant, reliable, human-derived and cost-effective cellular models is a present need to bring precision medicine to fruition and to accelerate drug discovery. 3D cultures show robust genomic stability and tissue-specific gene expression with high fidelity to their tissue of origin, making them ideal for preserving healthy and cancer tissue molecular and morphological traits.

We sought to establish and implement 3D cell-based assays suitable for detection of known molecular biomarkers in cancer using spherical colon cancer models (multicellular tumor spheroids and tissue-derived tumor spheres). We validate our procedures by detecting colorectal cancer (CRC) biomarkers both in patient-derived cultures and cell-lines (HCT116, HT29, LS174T). We expanded the study's scope to the analysis of specific CRC proteomic signatures in 3D.

We design, develop and validate cellular assays for human cancer 3D culture generation, long-term maintenance and analysis. We analyze and quantify cellular responses to cytotoxic drugs, image and quantify invasion in fluidic chambers, determine levels of viability.

METHODS

Our methodology takes advantage of long-term **imaging under controlled conditions** of temperature and gas levels (CO_2 and O_2). With this approach we can answer questions related to variations at the **transcription** and **protein level in 3D** cellular models and patient-derived samples.

To recreate physiological events we chose colon cell lines and tested them for their capacity to form lumens in a **3D microenviroment by matrix embedding**.

The impact of **cell adhesions in 3D (cell to cell, and cell to matrix)** in **drug cytotoxicity** was tested in **monolayers and spheroids**.

Spheroids are amenable to gene transcription variation measurements. We performed RT-PCR and detection of transcription levels of genes involved in hypoxic response and oncopathways.
We performed multiplex antibody microarrays to detect proteomic levels of metalloproteinases of which MMP-10 and MMP-9, as well as of surface proteins known to be involved in colorectal cancer invasion.

Alterations in **oxygen tension** are milestones in tumor progression. Low oxygen tension (hypoxia) has been demonstrated in CRC. The Hypoxia Inducible Factor (HIF) transcription factor family is central to the response to hypoxic stress and is correlated with CRC carcinogenesis, invasion, VEGF expression, and poorer diagnosis. To increase the relevance of our model we use hypoxic and normoxic conditions finding differences at the proteomic and transcriptional levels.

RESULTS

3D cultures recapitulate *in vivo* phenomena such as lumen formation and pathologic conditions such as metastasis
Recreating 3D microenvironments by matrix-embedding, co-culture with endothelial components and in association with microscopic analysis allows reliable insight on the chronology of normal and invasive events.

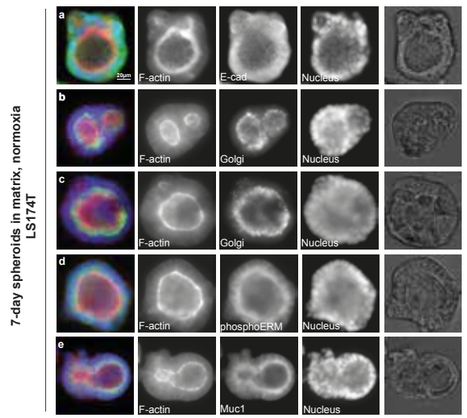
Microtissue emulation (spheroid cultures) will impact drug cytotoxicity
3D colon models in 3D culture display different response to drug doses compared to classical 2D cultures.

Transcription differences in drug response
We found that compared to normoxia and to controls, FOXC2 levels were reduced in hypoxia and with mitomycin C.
FOXC2 is known to be activated by hypoxia and VEGF, and to be a modulator of angiogenesis. These differential expression levels should be taken in account when planning assays with therapeutic drugs to assess concentration and exposure length.

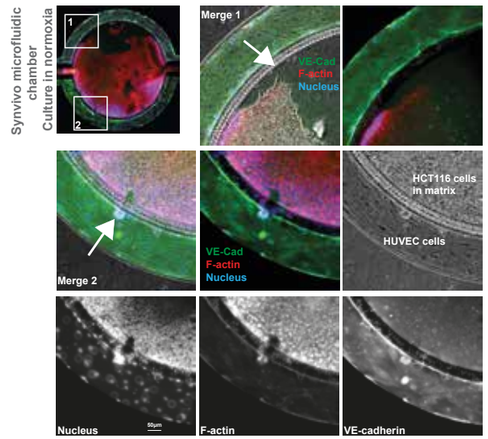
Matrix-tissue/tumor interactions are altered by hypoxic conditions
Colon cancer cellular models display differential TIMP1 secretion in 3D conditions (suspension) in hypoxia compared to normoxia (HT-29, HCT116 and LS174T spheroids).

3D cultures maintain morphological structure and function of tissue (healthy and tumor)
3D cultures of patient samples show differential MMPs profile in tumor and healthy tissues.

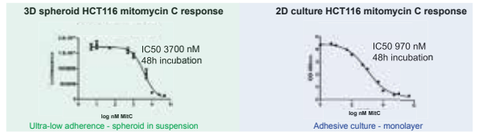
Colon adenocarcinoma spheroids acquire lumen-like morphology and cell polarity



Microfluidic chambers allow the observation of cell migration, change of nuclear shape and cytoskeleton organization during invasion of the endothelial compartment (HUVEC)



Cytotoxic response is altered in relation to culture conditions



Transcriptional changes in spheroids treated with mitomycin C

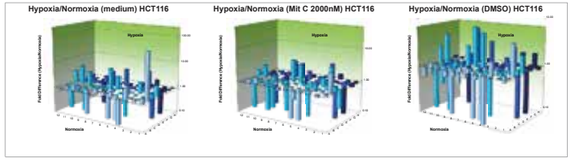
LS174T spheroids treated for 48h

Gene transcription variation DMSO/Mitomycin C

	Gene	Known Function	Fold-variation Hypoxia/ Normoxia
LS174T	CDC20 cell-division cycle protein 20	Cell cycle	3.8 fold down
	AURKB Aurora Kinase B	Centrosome separation and spindle attachment	3.7 fold down
	BCL2 B-cell lymphoma 2	Tumor suppressor	3.2 fold down
	MDM2 Mouse Double Minute 2	Inhibitor of p53; involved in cell death protection	4.64 fold up
	PLK4 Polo-like kinase 4	Cell cycle regulated centrosome duplication kinase	3.3 fold down
	PLK1 Polo-like kinase 1	Cell cycle	3.5 fold down

Oxygen levels influence gene expression (± mitomycin C)

HCT116 3D spheroids cultivated in hypoxia (3% oxygen) or at atmospheric oxygen levels



Gene transcription variation Hypoxia/Normoxia

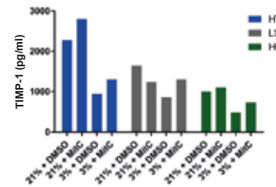
	Gene	Known Function	Fold-variation Hypoxia/ Normoxia
HTC116	CA9 Carbonic anhydrase IX	Catalizes reversible hydration of carbon dioxide	58 fold up
	IGFBP3 Insulin-like growth factor binding protein 3	Binds IGF-1 and IGF-2 with high affinity	3 fold up
	IGFBP5 Insulin-like growth factor binding protein 5	Binds IGF-1 and IGF-2; acts as tumor suppressor in melanoma	6 fold up
	KDR Kinase insert domain receptor	Cell surface receptor to VEGFA/C/D; regulates angiogenesis	3 fold up
	KRT14 Keratin14	Structural protein	6 fold up
	SERPINF1 Serpin peptidase inhibitor	Inhibitor of angiogenesis	5 fold up
	SLC2A1 Solute carrier family 2	Glucose transporter	4.5 fold up
	SNAI2 Snail2	Transcriptional repressor; EMT and apoptosis related	3 fold up

Gene transcription variation with mitomycin C, 2000nM, Hypoxia/Normoxia

	Gene	Known Function	Fold-variation Hypoxia/ Normoxia
HTC116	CA9 Carbonic anhydrase IX	Catalizes reversible hydration of carbon dioxide	3.8 fold up
	G6PD Glucose-6-phosphate dehydrogenase	Processing of carbohydrates; hypoxia induced	4 fold up
	FOXC2 Forkhead box family C2	Transcription Factor	3.7 fold down
	IGFBP3 Insulin-like growth factor binding protein 3	Binds IGF-1 and IGF-2 with high affinity	3.5 fold up
	HMOX1 Heme Oxygenase	Heme catabolism; mechanism of impact on metatstasis unclear	3.6 fold up
	SERPINF1 Serpin peptidase inhibitor	Inhibitor of angiogenesis	3.7 fold up
	SNAI2 Snail2	Transcriptional repressor; EMT and apoptosis related	3 fold up

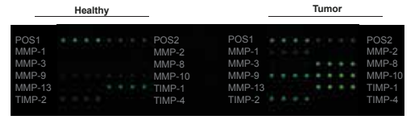
Hypoxic conditions (3% oxygen) impact on secreted levels of invasion-related protein TIMP-1

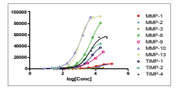
Oxygen Levels	TIMP-1 levels pg/ml HT29	LS174T	HCT116
21% + DMSO	2280	1640	1080
21% + MitC	2800	1240	1100
3% Oxygen + DMSO	904	860	490
3% Oxygen + MitC	1330	1300	735



3D cultures of patient-derived tissue are amenable to invasion-related secreted protein detection, namely MMPs and TIMPs using RayBiotech microarrays

Paired tumor and healthy tissue from a colon cancer patient





Biomarker	Tumor/Healthy
MMP-1 MMP-3 MMP-8 MMP-9 MMP-10 TIMP-1	Augmented secretion

CONCLUSION

We demonstrate that a combination of controllable assays in 3D cultures can successfully be used for identification of biomarker expression and compound screening in preclinical studies.

We are currently developing 3D models capable of reliably recapitulating physiological responses that are complementary to animal model assays.

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