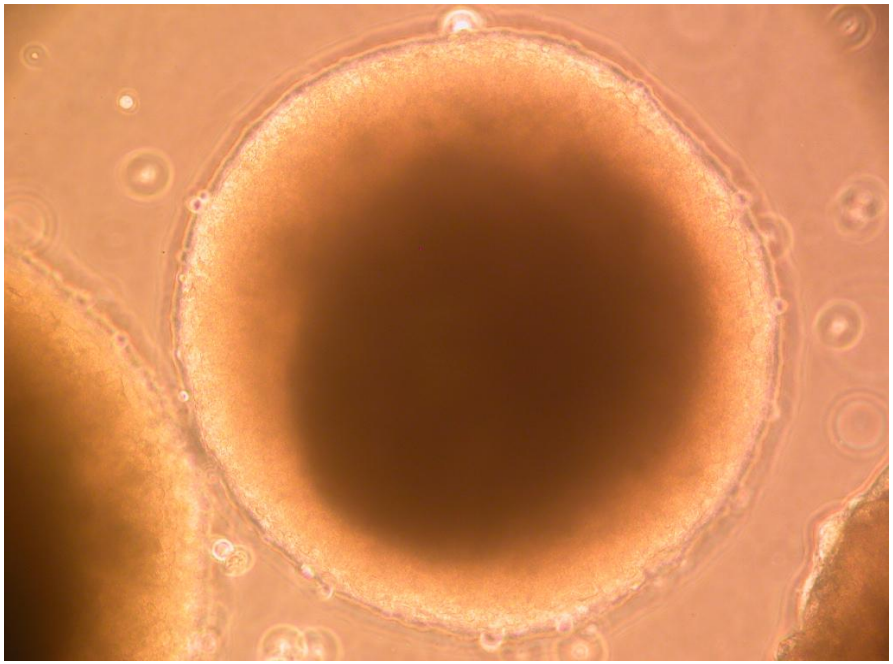
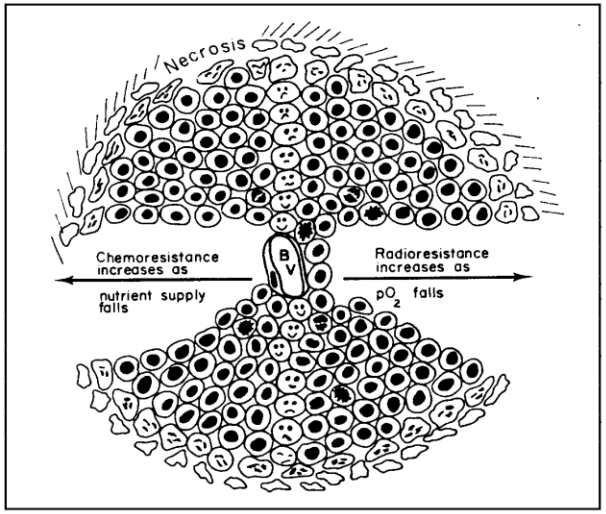
A comparison of proteins and their expression levels within different regions of multi-cellular spheroids (MCTS)

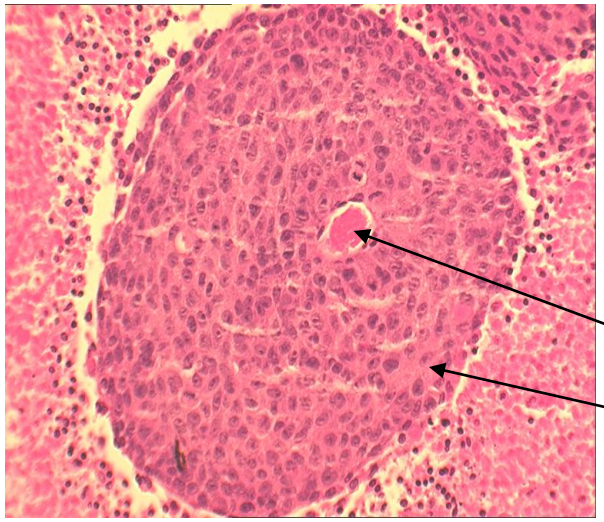


Kelly McMahon

Institute of Cancer Therapeutics

The tumour microenvironment





- Due to inefficient blood supply within tumours a ‘microenvironment’ is established.
- This occurs between 150 and 200 µm from a blood vessel (BV).
- Characteristic features of this include:
  - Hypoxia
  - Acidic extracellular pH
  - Low nutrient status
  - High levels of catabolites
  - Low cell proliferation rates
  - Biochemical adaptations
  - Beyond a certain distance, necrosis or cell death occurs.

Comparison of spheroid versus tumour

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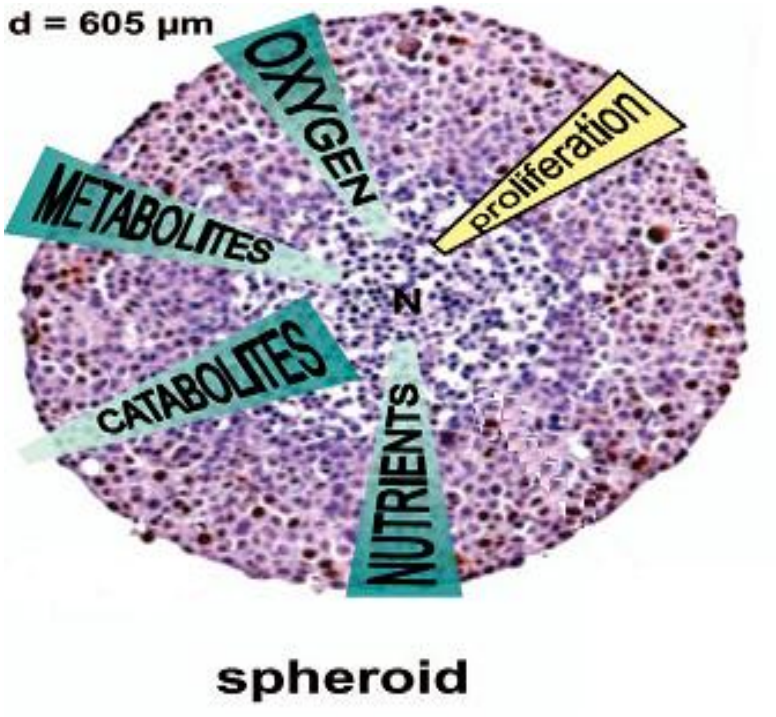

Histology * ECM * Cell adhesion

Cell signaling * Response to therapy

(Adapted from Kunz-Schughart, 1999)

Spheroid Separation

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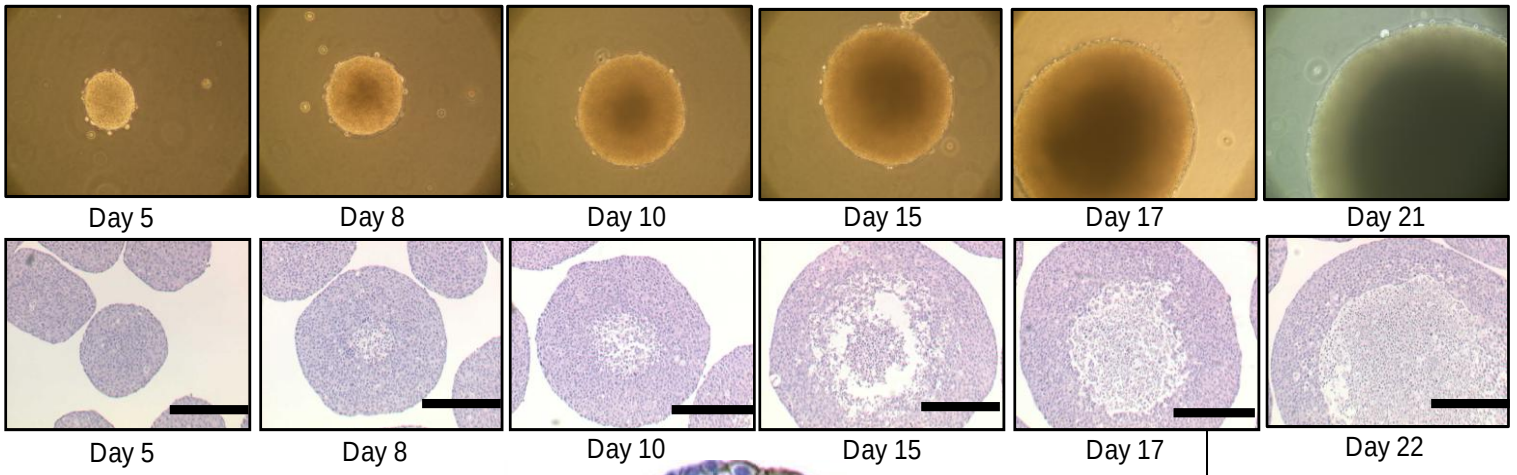
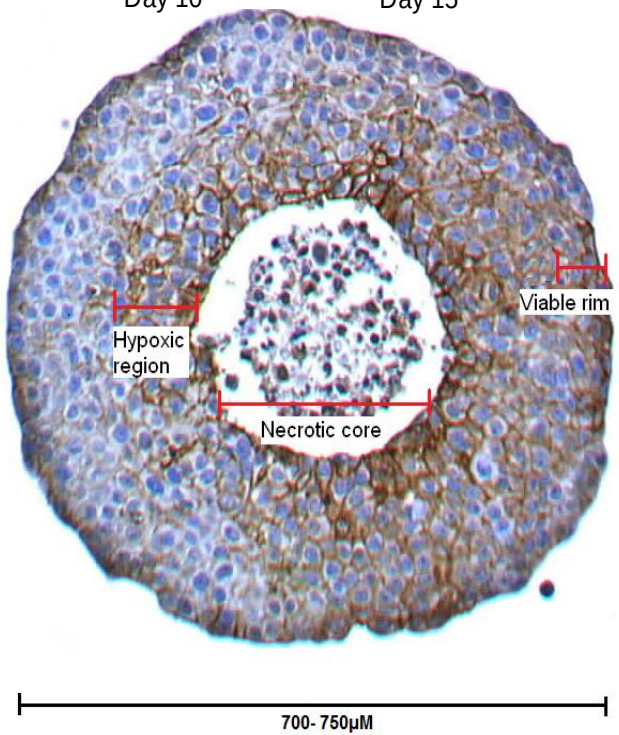


(Sutherland, Mccredie and Inch, 1971)



Spheroid Separation

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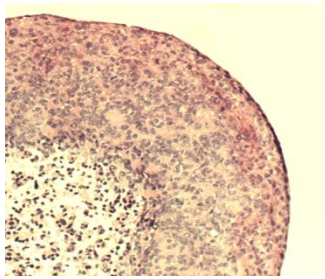

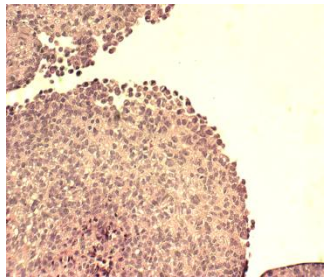

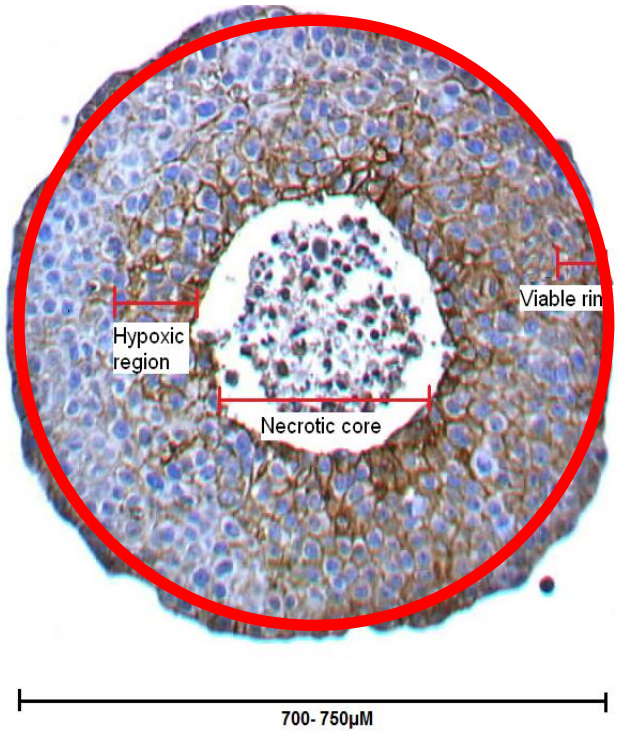

Spheroid Separation

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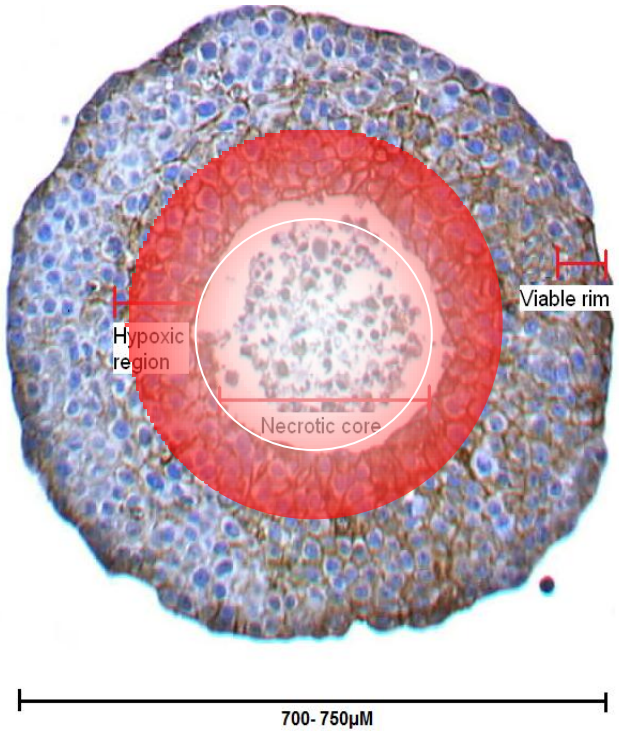

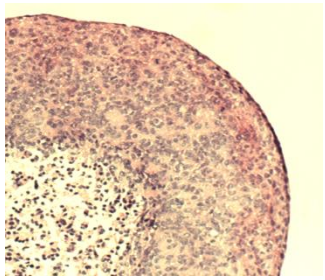

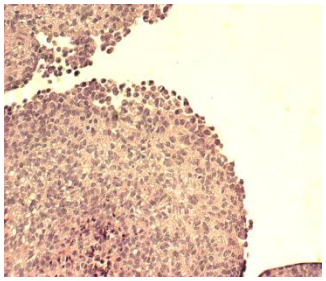

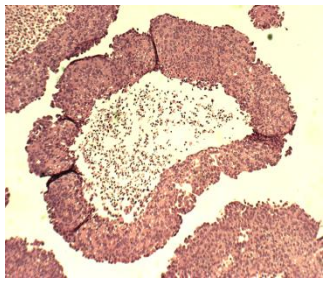

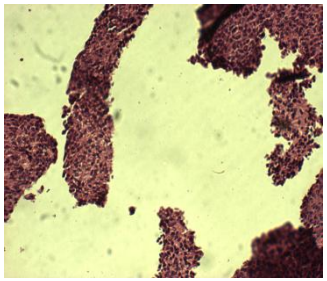

Spheroid Separation

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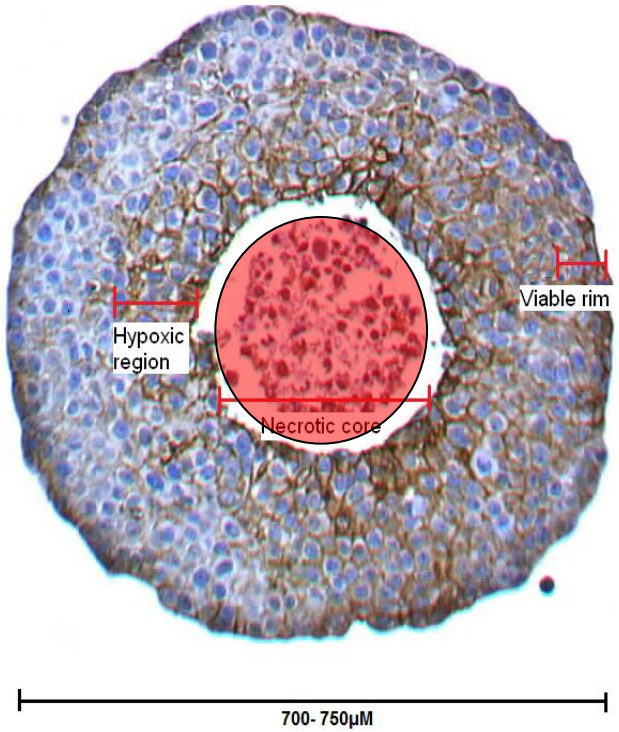

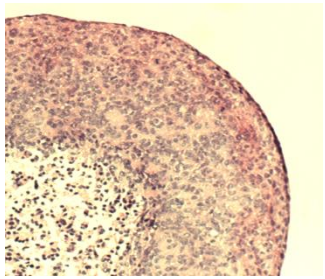

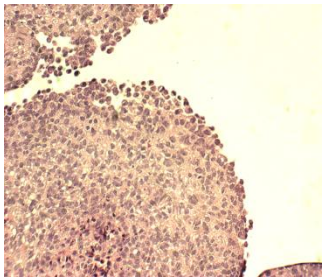

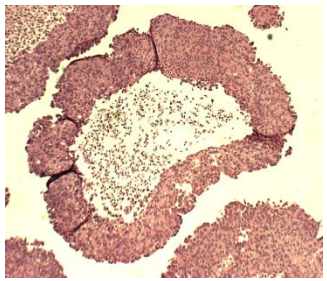

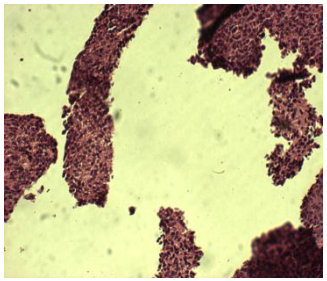

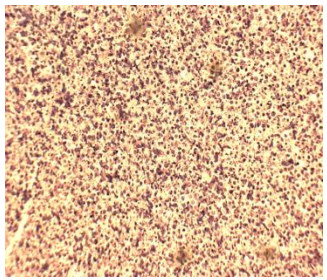

(Method adapted from Knowles and Phillips, 2001)

Validation of spheroid separation

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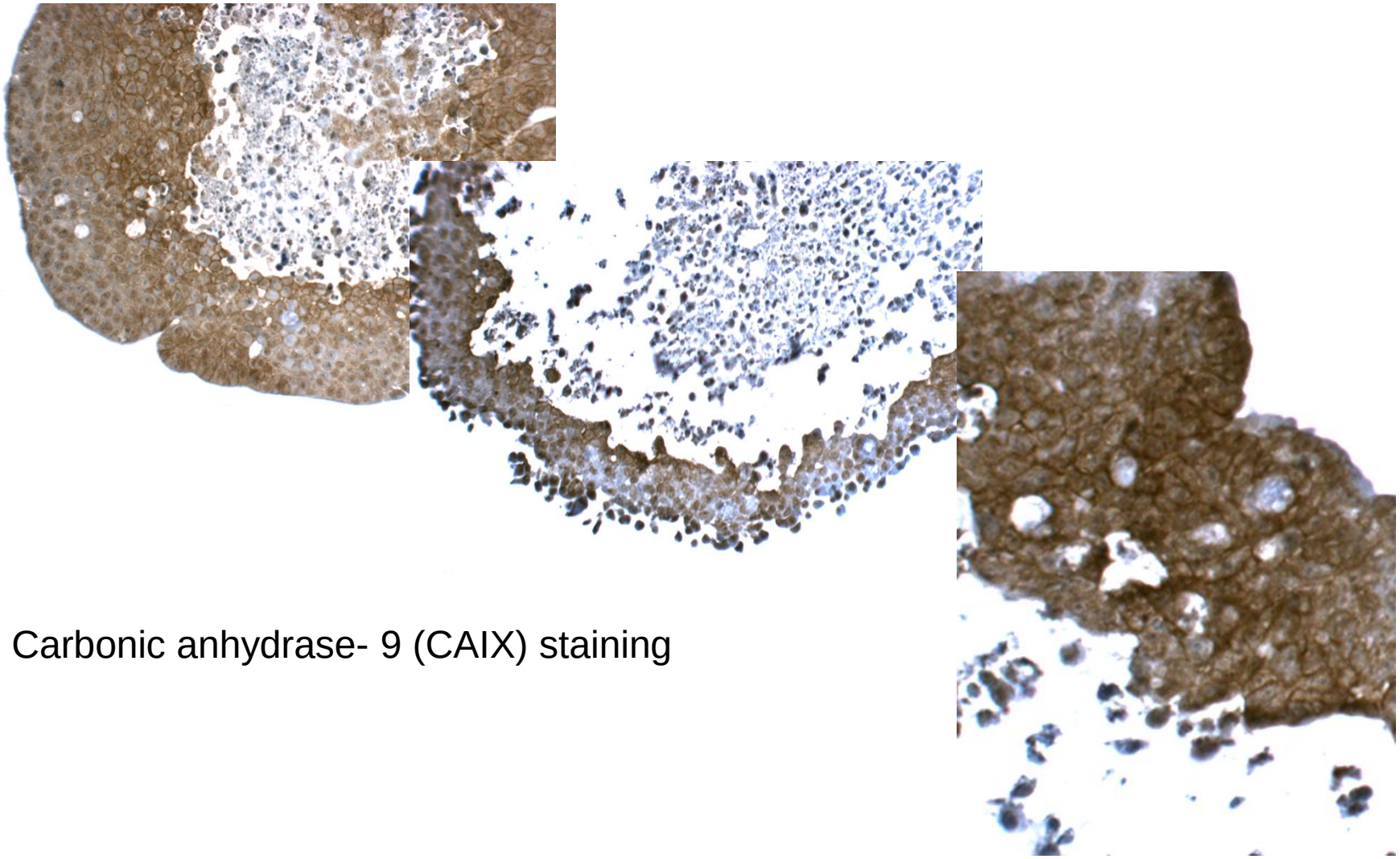

Carbonic anhydrase- 9 (CAIX) staining

Validation of spheroid separation

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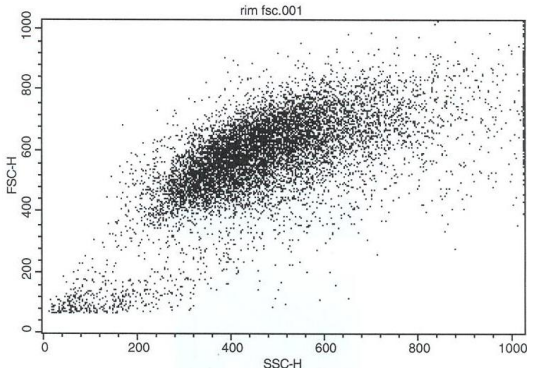
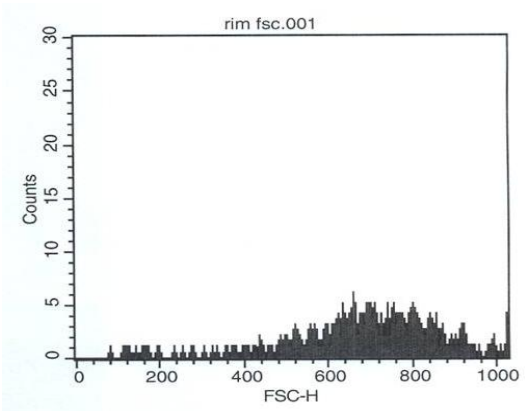
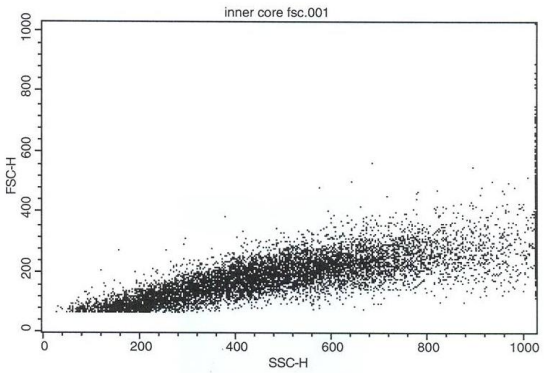
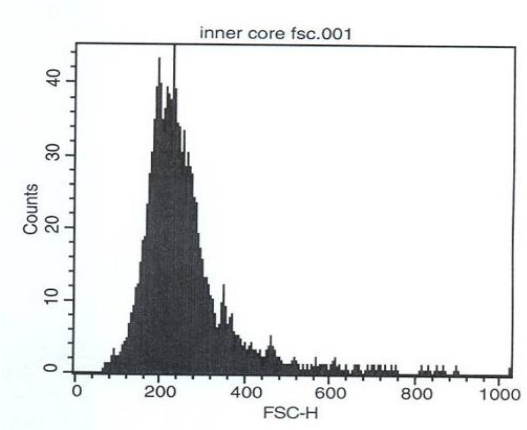
Viable rim



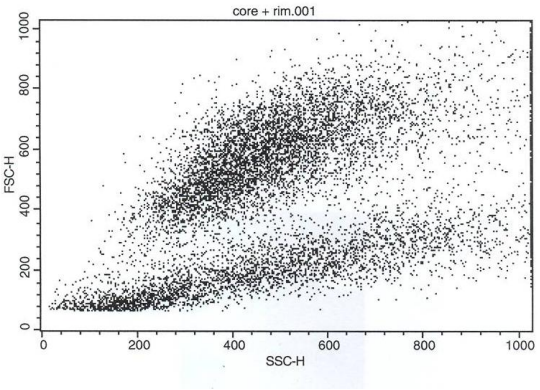


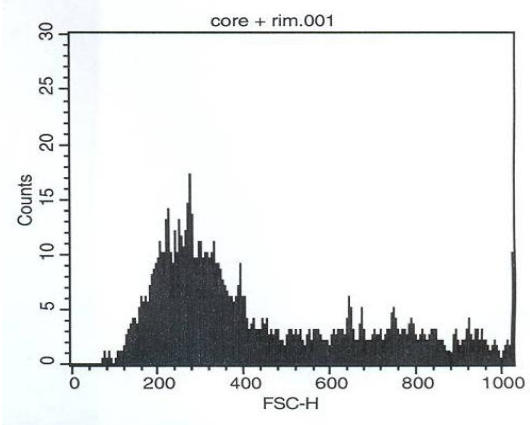
Necrotic core





Viable rim + necrotic core





FACS Analysis – Viable rim and necrotic core cells

Proteomics approach

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VR Inter HR NC

Cell lysis
sonication in the presence of lysis buffer

Protein isolation and purification
acetone precipitation

Protein concentration determination
Bradford assay

100µg 100µg 100µg 100µg

Reduction and Alkylation
50mM dithiothreitol (DTT) and
100mM iodoacetamide (IAA)

Trypsin
Digestion

114 115 116 117

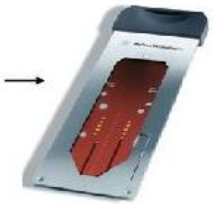
iTRAQ

OffGel Fractionation

Dionex RP nano HPLC system

MALDI fraction collector

Bruker Ultraflex II MALDI-TOF-TOF



Agilent 1260 HPLC-Chip system

Agilent 6530 Accurate-Mass Q-TOF LC/MS

Proteomics results

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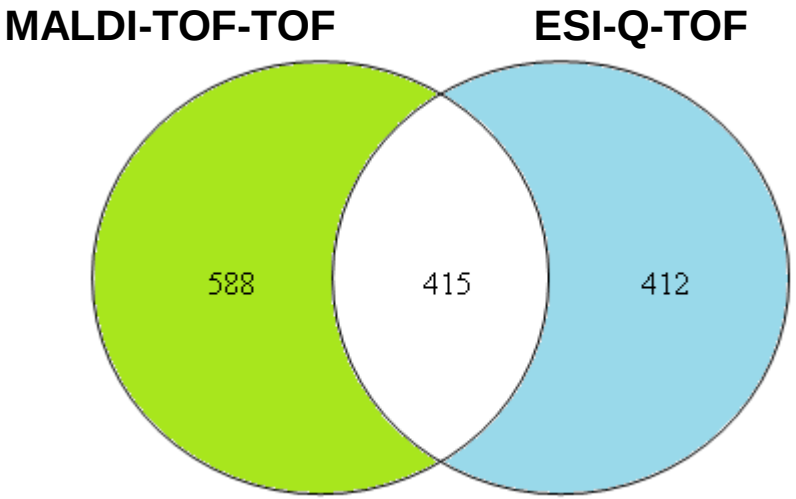

	Intermediate region		Hypoxic region		Necrotic core	
	MALDI-TOF-TOF	ESI-Q-TOF	MALDI-TOF-TOF	ESI-Q-TOF	MALDI-TOF-TOF	ESI-Q-TOF
Over expressed	156 (4)	120	111 (2)	166	185 (3)	97
Under expressed	116 (11)	206	74 (16)	173	77 (52)	245

Under and over expressed levels were those normalized ratio's which deviated plus or minus 1 standard deviation from the mean for that reporter ion, respectively.
(Proteins found differentially expressed with both instruments)

Glycolysis/Gluconeogenesis

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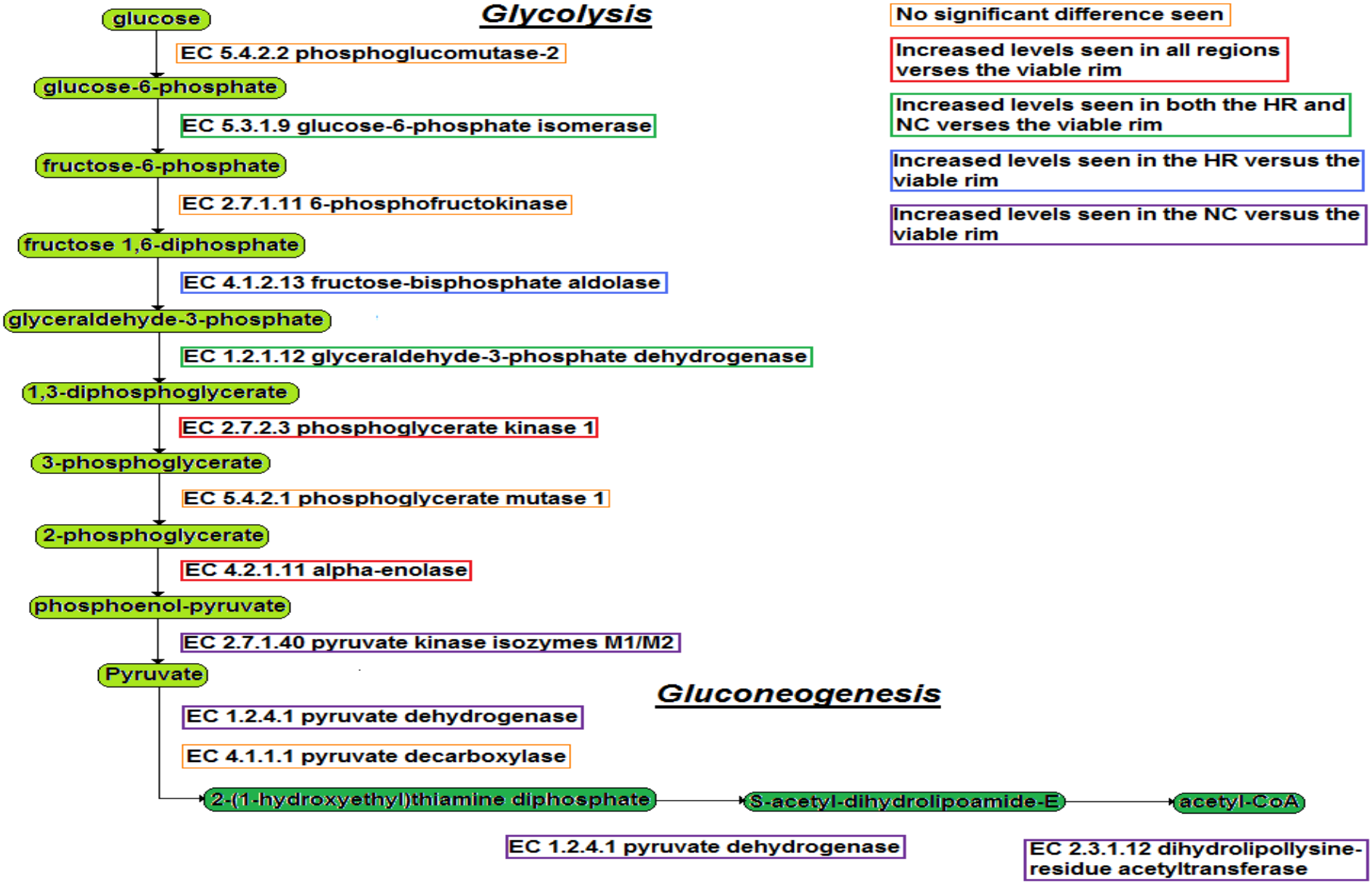

HS-1-associated protein X-1 (HAX-1)

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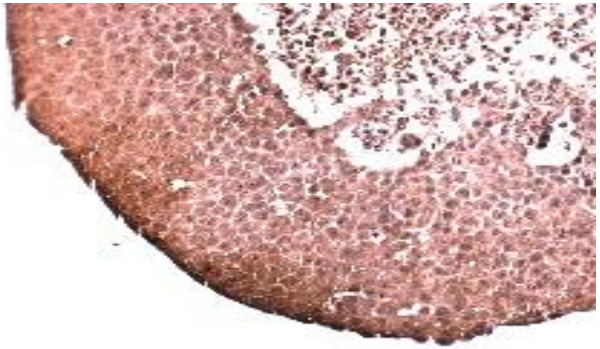
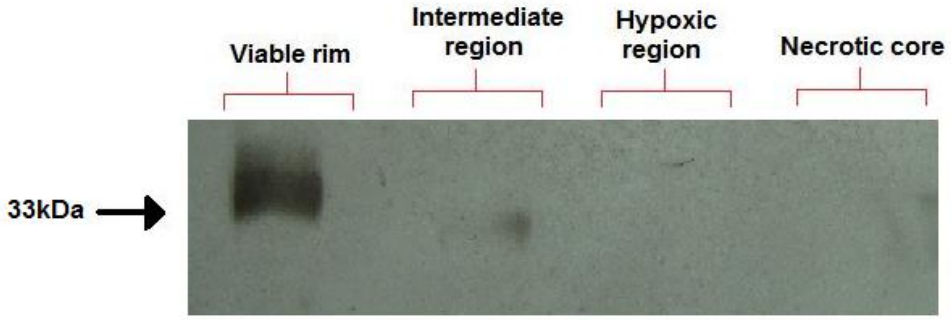


• Localised HAX-1 staining in the spheroids viable rim.



• Western blot analysis revealing a band in the viable rim sample only.

	114/115 Intermediate region	114/116 Hypoxic region	114/117 Necrotic core
Ratio	2.03 ↓	1.64 ↓	2.71 ↓

•These results both supporting the over expression of HAX-1 in the spheroids viable rim as first indicated by the proteomics analysis using iTRAQ.

Conclusions

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- The ability to fractionate spheroids into different regions for proteomics analysis, has been demonstrated
- Well established markers for hypoxia were up-regulated in the hypoxic region
- Changes occurring in the necrotic core are currently under investigation
- Proteins not previously association with hypoxia or cancer were shown differentially expressed

Ongoing work

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- Gene expression microarray analysis
- Continue validation of target proteins by Western blotting and immunohistochemistry
- Proceed with siRNA and functional assays for selected proteins

Acknowledgements

Institute of Cancer Therapeutics

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Roger Phillips
Sham Naal
Joachim Thiemann

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yorkshire cancer research