Extracellular matrix (ECM) proteins in hypoxia, 3D cancer spheroids as a model

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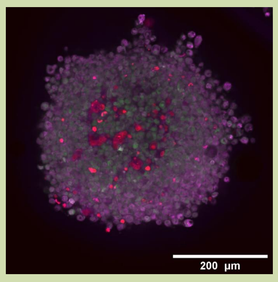
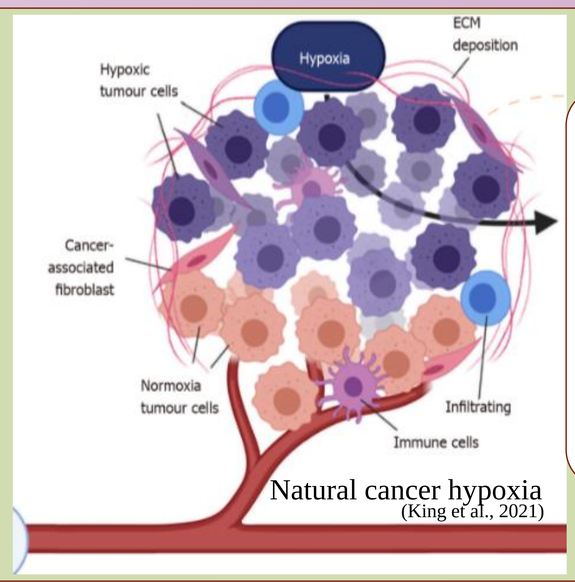


3D cancer spheroids in hypoxia

Introduction

Hypoxia (low level of oxygen) is a key factor in cancer growth. The disruption in oxygen homeostasis leads to extracellular matrix (ECM) remodeling and as a consequence, many ECM proteins are differentially expressed, and increased deposition is detected in tumor microenvironment (TME). This leads to alterations in ECM stiffness and cancer progression. In this study, 3D spheroids containing cancer cells and fibroblasts were used as they resemble complex cancer microenvironment better compared to 2D cell culture. Here we used spheroids to mimic cutaneous squamous cell carcinoma (cSCC) tumours.



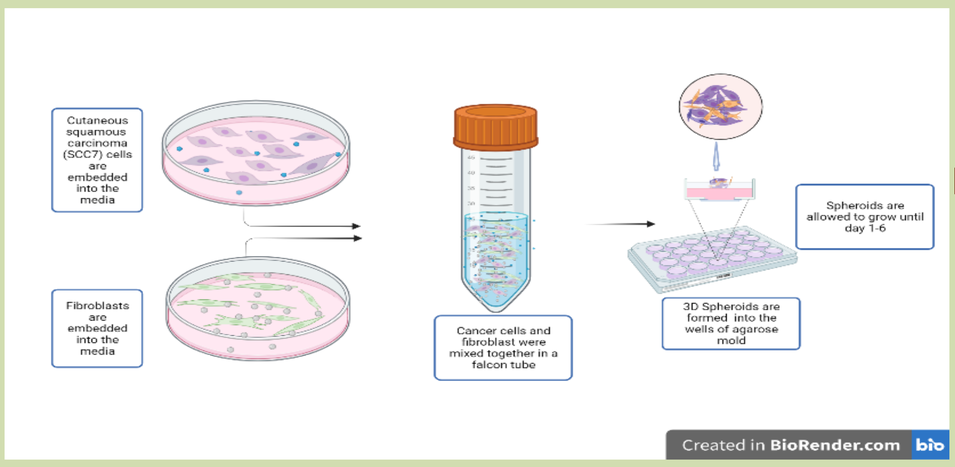
Natural cancer hypoxia (King et al., 2021)

1.ECM remodeling
2.Cancer invasion and metastasis
3.Treatment resistance

Aim of the study

1. Detection of ECM proteins : Collagen prolyl hydroxylases (P4HA1 & P4HA2), Lysyl hydroxylase (PLOD2), and glycoprotein Laminin-322 in cancer 3D spheroids in normoxia and hypoxia condition

Materials & methods



1. Western blotting
2. Proliferation assay
3. Immunofluorescence staining & Confocal imaging
4. Mass spectrometry analysis

Results

1. SCC7 cells and fibroblasts form tight spheroids both in normoxia and hypoxia

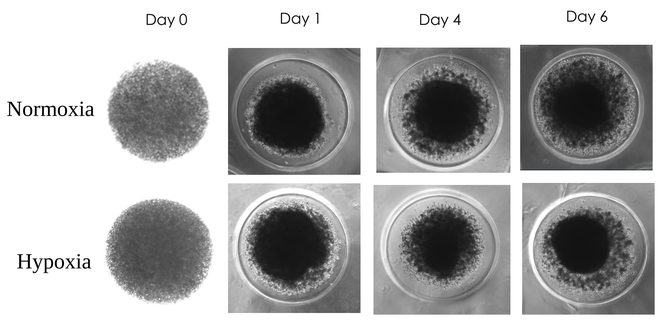
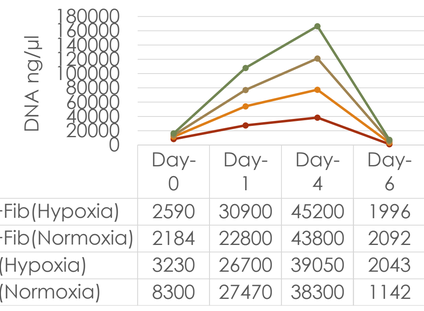


Figure 1: 3D cancer spheroids cultured in normoxia and hypoxia condition.

2.Cell proliferation was upregulated in hypoxia



	Day-0	Day-1	Day-4	Day-6
RT3+Fib(Hypoxia)	2590	30900	45200	1996
RT3+Fib(Normoxia)	2184	22800	43800	2092
RT3 (Hypoxia)	3230	26700	39050	2043
RT3 (Normoxia)	8300	27470	38300	1142

Figure 2: Cell proliferation assay of 3D cancer spheroids of transformed keratinocytes (RT3) with and without skin primary fibroblasts shows increased cell proliferation in hypoxia (3% O2).

3. Collagen prolyl hydroxylase (P4HA1 & P4HA2) and Lysyl hydroxylase (PLOD2) expression increases in co-cultured spheroids in hypoxia

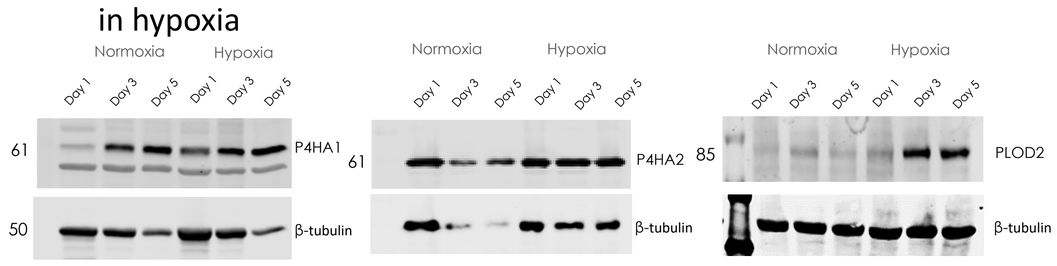


Figure 3: Western blot was used to detect collagen prolyl hydroxylases (P4HA1 & P4HA2) and lysyl hydroxylase (PLOD2) in 3D spheroids (RT3 cell line with skin primary fibroblasts). Beta tubulin was used as a loading control.

4. Laminin-332, P-Smad2, P-Creb and P-ERK1/2 expression in co-cultured cancer 3D spheroids in normoxia and hypoxia

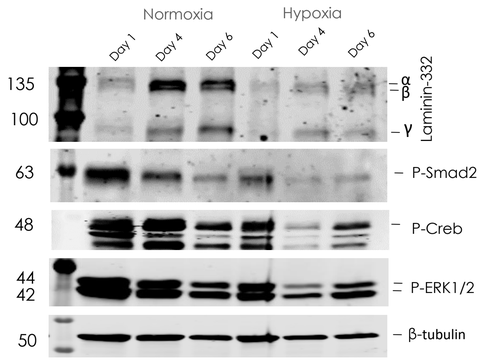


Figure 4: Westernblot was used to detect laminin-332, P-Smad2, P-Creb and P-ERK1/2 expression in cancer 3D spheroids in hypoxia. Beta tubulin was used as loading control.

5.P4HA1 showed enhanced expression in 3D spheroids in hypoxia

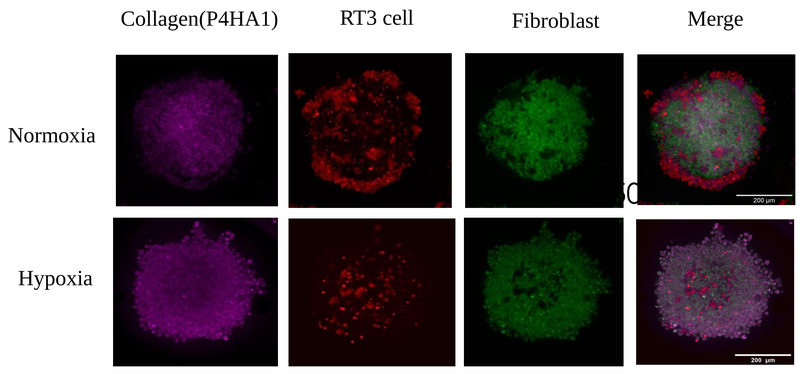


Figure 5: 3D spheroids containing RT3 cells and skin primary fibroblasts were stained with collagen prolyl hydroxylase (P4HA1) antibody and images were taken with confocal microscope. Result shows increased P4HA1 expression in hypoxia. Scale bar 200µm.

Conclusions

1. These results suggest that three of the core ECM proteins, namely collagen prolyl hydroxylases (P4HA1 and P4HA2) and collagen lysyl hydroxylase (PLOD2) expression increased in hypoxia both in transformed keratinocytes and metastatic cSCC (cutaneous squamous cell carcinoma) spheroids when these cell lines were cultured with human primary skin fibroblasts.
2. Hypoxia induces cell proliferation in cSCC .