In Vivo biomarker expression patterns are preserved in 3D cultures of Prostate Cancer.

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Introduction

Research investigating the cellular and molecular differences in Prostate Cancer (PCa) cells cultured in traditional 2D versus 3D assays is limited. Of these, comparative analysis has been primarily based on gene expression and microarray data[1]. Here we have undertaken direct comparisons between 2D and 3D, temporally comparing expression of biomarkers and cell adhesion molecules known to be associated with metastatic dissemination in PCa.

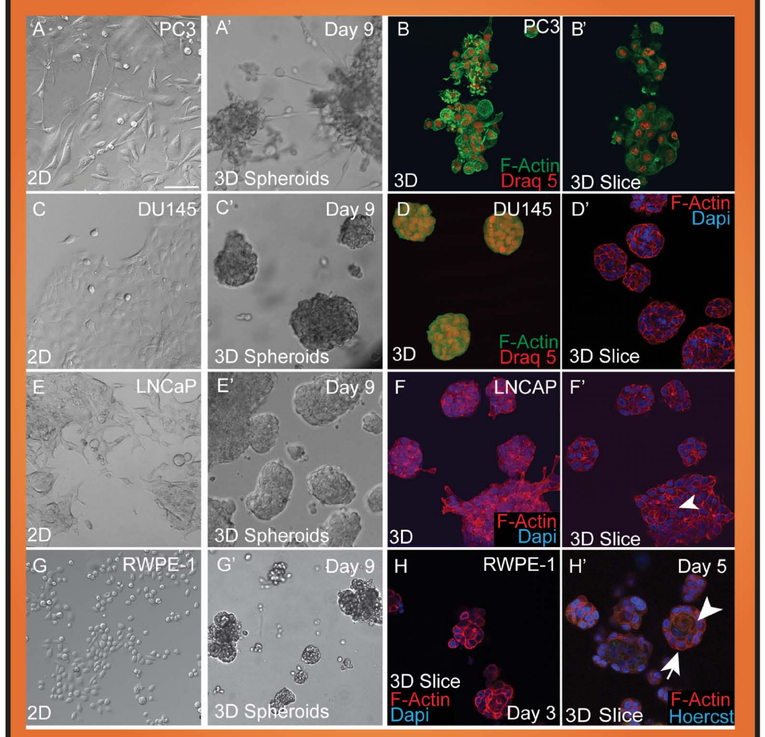
Antigenic profiles of tumours excised from advanced PCa patients have identified alterations in the expression of numerous proteins. Of these, the Androgen receptor (AR), α6 integrin and β1 integrin subunits, Vimentin, the cell adhesion molecules, E-Cadherin and N-Cadherin and more recently chemokine receptor CXCR4 expression have been linked to increased Gleason grade and metastatic dissemination in PCa[2-7].

Using these well established markers we present novel data concerning the direct differences in protein expression exhibited by a range of PCa cell-lines using both traditional 2D monolayer versus 3D culture systems. Our results indicate that 3D cultures afford a better platform that discerns antigenic profiles which more directly mimic that found in vivo.

Results

1. Cell morphology of PCa cells lines in 2D and 3D cultures.

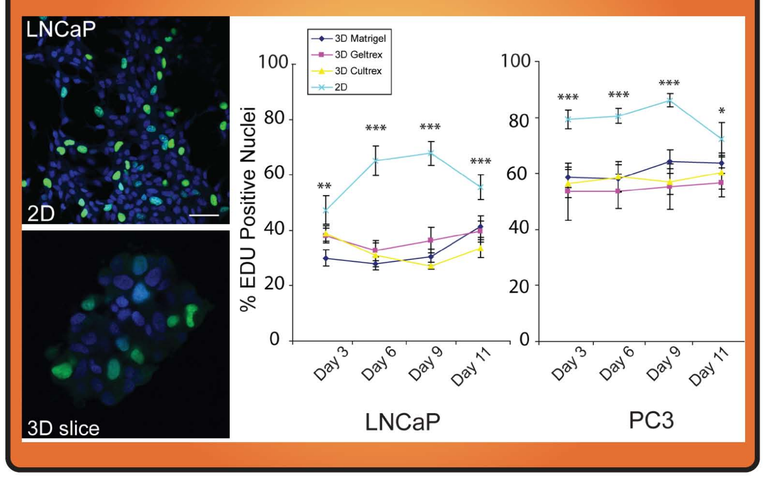
We analysed the distribution and expression profiles of E-cadherin and N-cadherin between metastatic PC3 and the epithelial cell line RWPE-1 in 2D and 3D cultures across a 9 day time-course via immunocytochemistry, Acapella script-based image analysis and western blot techniques.



Non-invasive LNCaP (F-F') and metastatic cell lines PC3 (B-B') and DU145 (D-D') form amorphous irregular shaped colonies that lack the polarised organisation of true acini as found in prostate epithelial cell line RWPE-1 (H-H' arrowhead)

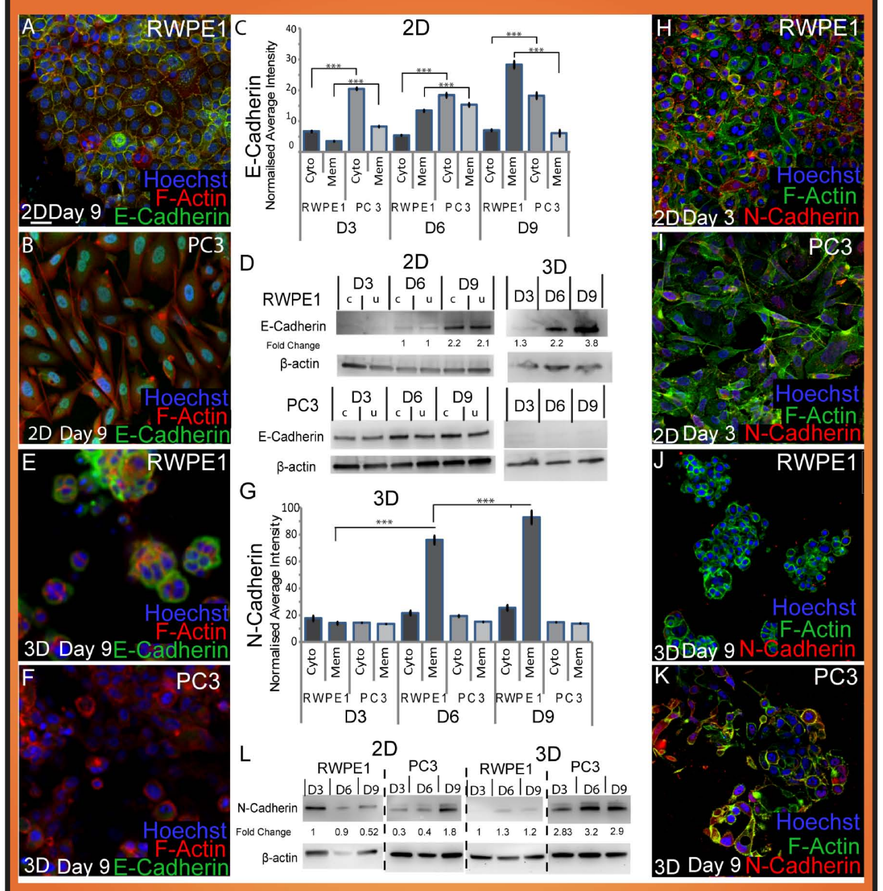
2. Proliferation rates are artificially enhanced in 2D

We analysed the proliferation rates (EDU click-it) of PCa cell-lines cultured in 2D and 3D cultures. In all instances PCa cells plated in a 3D matrix were found to proliferate at significantly lower rates than those plated in 2D cultures.



3. Altered E-cadherin and N-cadherin expression in 3D PC3 cells.

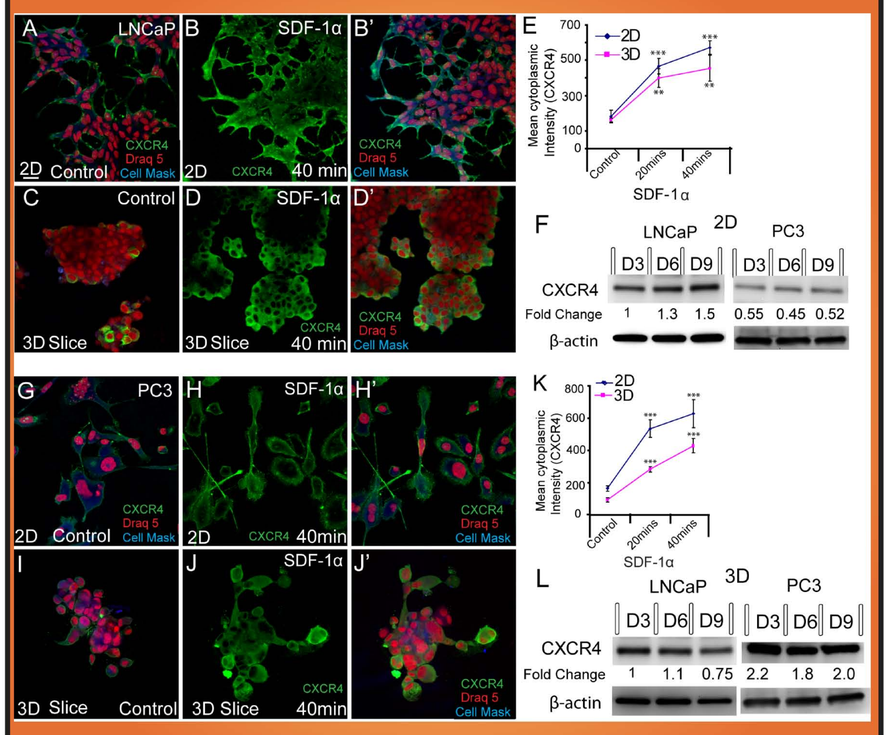
We analysed the distribution and expression profiles of E-cadherin and N-cadherin between metastatic PC3 and epithelial cell line RWPE-1 in 2D and 3D cultures across a 9 day time-course via immunocytochemistry, Acapella script-based image analysis and western blot techniques.



In comparison to RWPE-1 cells in 2D (A, D), in 3D, RWPE-1 cells up-regulated expression of E-cadherin with the distribution associated predominantly with cell-ECM contacts (E, D). By contrast, in comparison to their 2D counterparts (B, D) PC3 cells grown under 3D conditions lost all expression of E-cadherin (F, D) and up-regulated N-cadherin (K, L) which more closely resembles the expression pattern of metastatic cells in vivo and the observations regarding a loss of E-cadherin in cancer cells. In 2D, similar protein expression levels of N-cadherin and E-cadherin were observed indicating little discrimination between normal and metastatic cell-lines.

4. Up-regulation of CXCR4 in metastatic PC3 in 3D

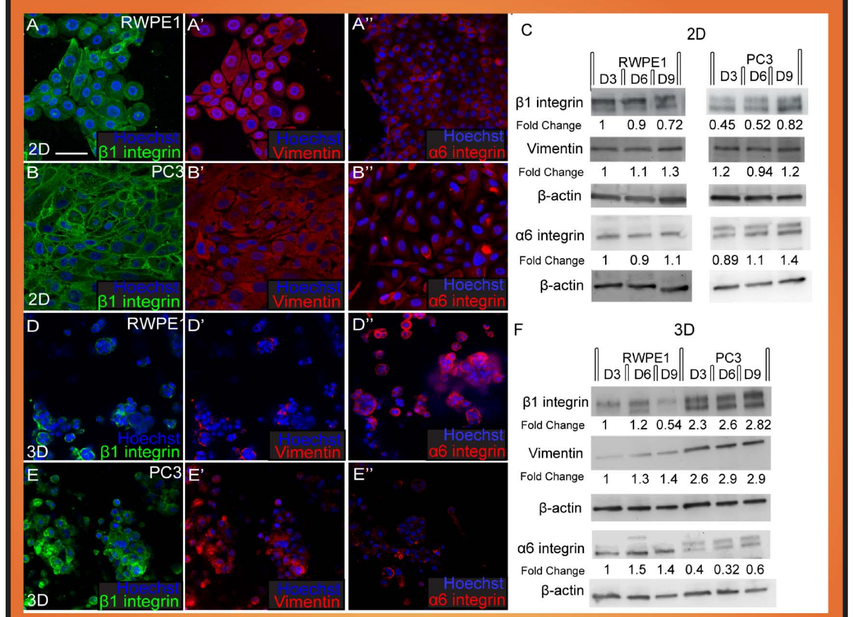
Differences in expression and function of CXCR4 in 2D and 3D cultures in non-invasive LNCaP and metastatic PC3 cells was investigated In the first instance we established whether CXCR4 was functionally active in these cell-lines via ligand stimulation with SDF-1α.



In 2D cultures, non-invasive LNCaP cell lines were found to express higher protein levels of CXCR4 in comparison to metastatic PC3 cells (F). Alternatively, in 3D cultures PC3 cells were found to express CXCR4 at a two fold increase when compared to LNCaP cells (L), consistent with metastatic disease progression and development as reported by clinical samples.

5. Altered Vimentin, β1 and α6 integrin expression in 3D PC3 cells

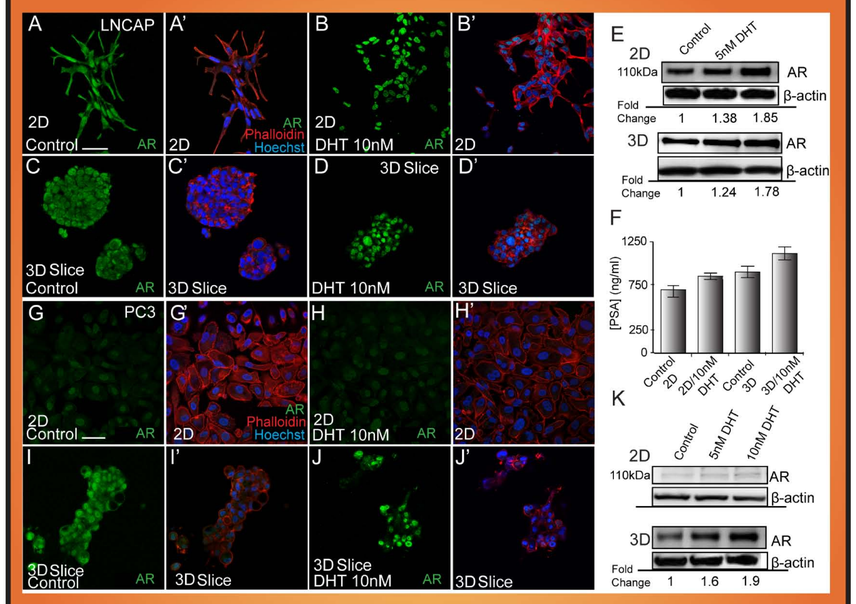
To determine the relative expression of Vimentin, β1 and α6 integrin markers in 2D vs 3D cultures, we investigated PC3 and RWPE-1 cells across a 9 day time-course via immunocytochemistry and western blot techniques.



In comparison to their 2D counterparts, in 3D, both β1 integrin and Vimentin expression was clearly up-regulated in PC3 cells (E-E') when compared to RWPE-1 cells (D-D'). Densitometric results confirmed a two fold increase in the total protein expression of both β1 integrin and Vimentin expression in PC3 cells (F). In 3D cultures, α6 integrin was distributed primarily on the membrane of both RWPE-1 (D'') and PC3 cells (E'') where it is known to functionally interact with ECM. Consistent with clinical samples, PC3 cells exhibited a down-regulation of α6 integrin protein expression across all days in culture (Fig. 4F).

6. Up-regulation of endogenous AR in 3D

We investigated the effect of DHT on AR function and expression on androgen-responsive LNCaP cells and androgen-refractory cell lines DU145 and PC3 in 2D and 3D cultures. Using a combination of immunocytochemistry,western blot and PSA ELISA techniques, we were able to establish AR distribution and functionality in these cell-lines.



LNCaP cells expressed high levels of endogenous AR n both 2D (A-A') and 3D (C-C'). In response to 10nM DHT, nuclear translocation of AR was evident in 2D (6B-B') and 3D (D-D'). AR protein expression increased in the presence of 10nM DHT (E). PSA ELISA confirmed that AR in LNCaP cells is transcriptionally active and functional in both 2D and 3D cultures (F). In 2D, PC3 cells did not express AR (G-G') and was not responsive to DHT treatment (H-H'). In 3D, PC3 cells re-expressed AR (I-I') and was responsive to DHT treatement with nuclear translocation evident (J-J'). Western blot results confirmed these results (K). PSA ELISA results revealed that AR was not transcriptionally functional in these cells.

Conclusion

In summary, we have developed and optimised 3D cell culturing techniques for a range of PCa cell-lines. We have shown that cell morphology, proliferation rates and biomarker expression are altered significantly between non-cancerous epithelial, non-invasive and metastatic PCa cell-lines when grown in 3D culture, with 3D cultures mimicking more precisely cancer progression as reported *in vivo*. As such, 3D models of PCa may serve as a more biologically relevant in vitro model and intermediate step in the drug discovery pipeline.

Acknowledgements

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