Abstract # 263944_1

Regulation of the Chemokine Receptors CXCR7 and CXCR4 in 3D Culture Models of Prostate Cancer

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Introduction

Prostate cancer (PCa) is known to be highly regulated by the chemokine stromal derived factor 1-alpha (SDF-1α) and its receptor CXCR4, however the alternative SDF-1α-binding receptor CXCR7 has also been found to regulate cell survival and invasion [1]. Whilst expression of CXCR7 is highly restricted in non-malignant cells, it is widley expressed in many different tumour cell lines [2]. *In vivo* prostate tumour biopsies show a pattern where CXCR7 expression increases with invasive grade, as previously reported for CXCR4 [1]. However, there is limited knowlege on the role of CXCR7 and its function in PCa. In this study, we aim to characterise the regulation of cell growth and behaviour by CXCR7 in PCa. The regulation of both CXCR7 and CXCR4 in three-dimentional (3D) culture models of PCa cell lines will be investigated to assess how the formation of tumour-like spheroids affect receptor expression and function. Further elucidation of CXCR7 function, with respect to CXCR4, will shed light on how these receptors contribute to regulation of the metastatic process in PCa - known to be heavily regulated by CXCR4.

Methodology

Western Blotting: Cells were cultured in 2D on TC-coated dishes (2D) or on a thin layer of 70% Matrigel [BD Biosciences]. Samples were lysed in RIPA buffer, run on SDS-PAGE then western blot. Primary antibodies used were (Rabbit anti-huCXCR7 [ab72100; Abcam], Rabbit anti-huCXCR4 [ab2074; Abcam], Mouse anti-β Actin [Sigma-Aldrich]). We used HRP-conjugated secondary antibodies [Bio-Rad], Western Lightning ECL Substrate [Perkin Elmer], and the Versa Doc Imaging Station [Bio-Rad].

Immunofluorescence: Primary antibodies used were Mouse anti-CXCR7 9C4 [MBL International] alone, or Rabbit anti-CXCR7 [Abcam] along with Mouse anti-CXCR4 [R&D systems] for colocalisation studies. Secondary antibodies were Rabbit anti-mouse Alexa Fluor 488 or Goat anti-rabbit Alexa Fluor 594 [Invitrogen, Life Technologies]. CellMask Blue [Invitrogen] was used, cells imaged on Olympus IX-81 Scanning Confocal microscope.

Proliferation Assay: Cells were cultured in TC-treated 384 well plates [BD Falcon] for up to 10 days, media replenished every 2-3 days. At indicated time, Alamar Blue was applied directly into wells at a final concentration of 10% (v/v), incubated for 4h at 37°C and read on Envision Plate Reader [Perkin Elmer].

Chemokine receptors in 2D cultures

Less invasive cell line expresses highest level of CXCR7: Endogenous expression of CXCR7 and CXCR4 were assessed across various cell lines derived from normal and cancerous prostate epithelium in 2D culture. Interestingly, the less invasive LNCaP cells expressed much higher levels of these receptors which are associated with invasive behavoiur (Fig. 1A). Upon treatment, we found the CXCR7 receptor to be ligand-responsive through internalisation from the membrane and cytpolasm into punctate structures in the cytoplasm (LNCaP, Fig.1B)

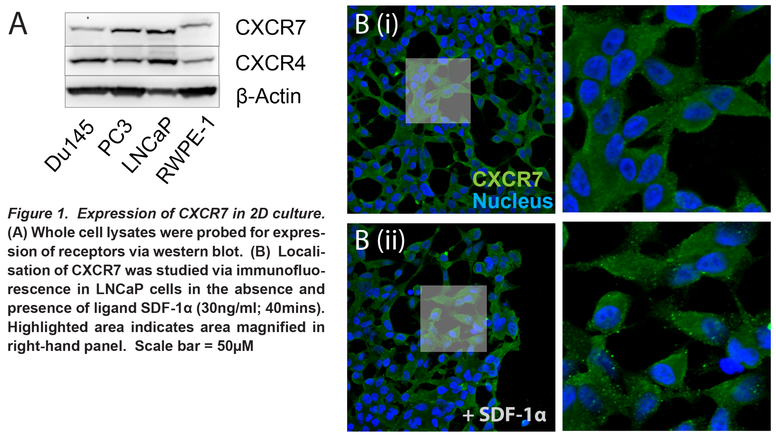

Figure 1. Expression of CXCR7 in 2D culture. (A) Whole cell lysates were probed for expression of receptors via western blot. (B) Localisation of CXCR7 was studied via immunofluorescence in LNCaP cells in the absence and presence of ligand SDF-1α (30ng/ml; 40mins). Highlighted area indicates area magnified in right-hand panel. Scale bar = 50μM

Reduced cell proliferation by CXCR7 inhibition: Inhibition of CXCR7 had a negative impact on cell proliferation in the androgen-dependent LNCAP cell line when cultured in depleted media. Inhibition of CXCR7 had no observable effect on the growth of RWPE-1, PC3 and Du145 cell lines in either condition. This suggests that reliance on CXCR7 for cell growth/survival differs between PCa cell lines. Further, this potentially suggests depletion of androgens sensitises androgen-dependent cells (LNCaP) to CXCR7 inhibition.

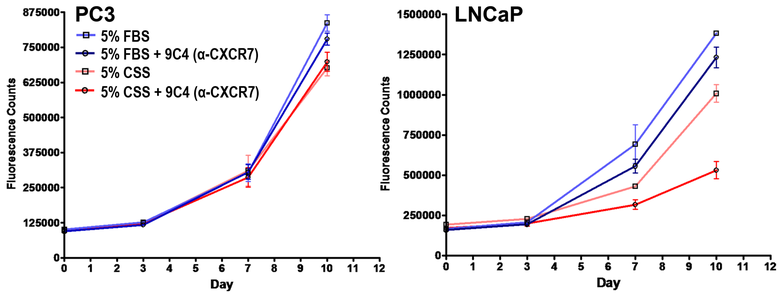

Figure 2. Regulation of cell growth by CXCR7. Cells were plated and treated for the indicated length of time in either 5% FBS or 5% Charcoal-stripped FBS, prior to incubation with Alamar Blue and reading fluorescence signal on the Envision Plate Reader (Perkin Elmer).

Differential regulation of chemokine receptors in 3D cultures

Chemokine receptor expression changes during spheroid development: CXCR7 and CXCR4 are known to be regulated inversely during embryonic development, and CXCR7 can regulate tumour development *in vivo*, so the expression of these receptors was assessed during spheroid development in 3D culture. We observed an upegulation of both receptors in PC3 and the normal epithelial RWPE-1 cell line whilst other cell lines were unchanged (Fig. 3). This upregulation in 3D culture occured at days 7-10 when the spheroids are more well-formed with a higher degree of cell to cell contact.

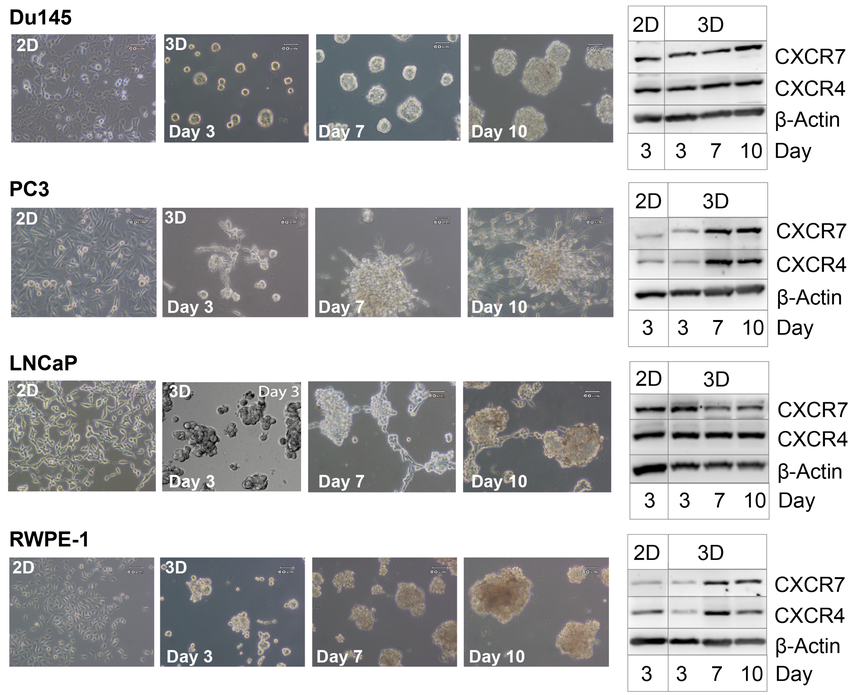

Figure 3. Expression of CXCR7 and CXCR4 over time in 3D culture. DIC images of 2D cultures and 3D spheroids are shown on left, and right hand panels show whole cell lysates probed on western blots for receptor expression.

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References: [1] Wang, J. et al (2008) The Role of CXCR7/RDC1 as a chemokine receptor for CXCL12/SDF-1 in Prostate Cancer. The Journal of Biological Chemistry. 283; 7. p4283-4294. [2] Burns, J. et al (2006) A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion,. and tumour development. The Journal of Experimental Medicine, 203; 9. p2201-2213

Ligand-stimulated colocalisation of receptors in 3D cultures

Response to SDF-1α results in receptor colocalisation in 3D culture: Using immunofluorescence, a receptor internalisation response was visualised in 3D cultures of LNCaP cells, thus confirmng that 3D cultures are ligand-responsive (Fig. 4A). A distinct colocalisation of CXCR4 and CXCR7 was observed in ligand-treated 3D cultures, indicating dual internalisation of both receptors (Fig 4B i-ii).

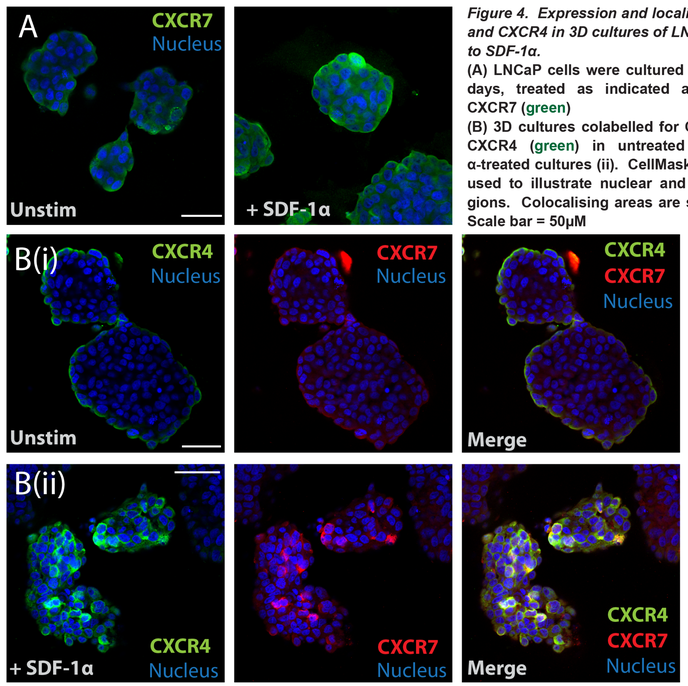

Figure 4. Expression and localisatoin of CXCR7 and CXCR4 in 3D cultures of LNCaP in response to SDF-1α.
(A) LNCaP cells were cultured in Matrigel for 6 days, treated as indicated and labelled for CXCR7 (green)
(B) 3D cultures colabelled for CXCR7 (red) and CXCR4 (green) in untreated (i) and SDF-1 α-treated cultures (ii). CellMask Blue (blue) was used to illustrate nuclear and cytoplasmic regions. Colocalising areas are shown in yellow. Scale bar = 50μM

PLEASE NOTE: Here CXCR7 shown in red and CXCR4 in green

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Summary

1. Culturing of normal prostate epithelial or PCa cells in 3D matrices results in upregulation of chemokine receptors in some cell lines
2. CXCR7 was observed to regulate cell growth in androgen-depleted conditions in androgen-dependent PCa cell line only.
3. This suggests that there is differential regulation of CXCR7 between prostate cell lines which perhaps reflects the heterogenous nature of tumour cell populations