Investigating the role of protein kinase D (PKD) in chemokine signaling via CCL3-CCR5 axis

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Introduction

The crosstalk between normal cells and cancer cells in the tumour microenvironment contributes to the spread of cancer cells[1]. Chemokines are essential mediators between cells by interacting with chemokine receptors overexpressed in cancer cells[2]. A downstream signalling protein, protein kinase D (PKD), possesses a critical regulatory role contributing to diverse effects in cell migration among different cell lines in chemokine signalling[3]. **Aim** - to investigate differentiate mechanisms underlying the regulatory role of PKD in THP-1 leukaemic monocyte cells and MCF-7 breast cancer cells in CCL5-CCR3 axis.

For more details on signalling pathway:

Methods

Experimentation using two different pan-PKD inhibitors, CID755673 and CID2011756.

- **Immunofluorescence** using anti-CCR5 antibody derived from HEK/1/85a/7a cell growth supernatant and probed with anti-rat ALEXA 488.
- **Calcium Flux Fura-2AM Assay** CCL3-induced Ca^{2+} release in inhibitor-treated cells compared with untreated control.
- **Data Analysis:** Analysis performed in GraphPad Prism using One Way ANOVA by post-hoc Bonferroni tests $p< 0.05$ (*) $p < 0.0001$ (***) $p<0.0001$ (****)

Results

Table 1: Immunofluorescence – percentage of chemokine receptors compared to control 100nM CCL3, n≥ 3

Cell line	Percentage of cell surface chemokine receptors				
	CCL3	CID2011756		CID755673	
		Without CCL3	With CCL3	Without CCL3	With CCL3
MCF-7	26.9 ± 2.5	80.2 ± 6.1	74.7 ± 3.4****	86.4 ± 12.3	65.4 ± 6.0***
THP-1	47.3 ± 4.9	86.1 ± 15.2	104.0 ± 16.6*	91.3 ±13.1	82.8 ± 13.4

PKD is potentially involved in CCL3-induced CCR5 internalisation in both cell lines.

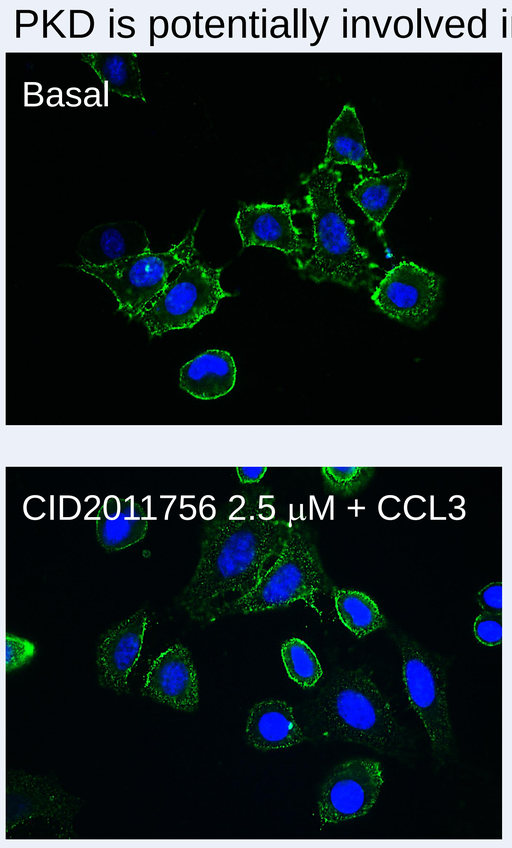

Figure 1: CCR5 receptor internalisation in MCF-7 cells stimulated by CCL3 is blocked by both PKD inhibitors

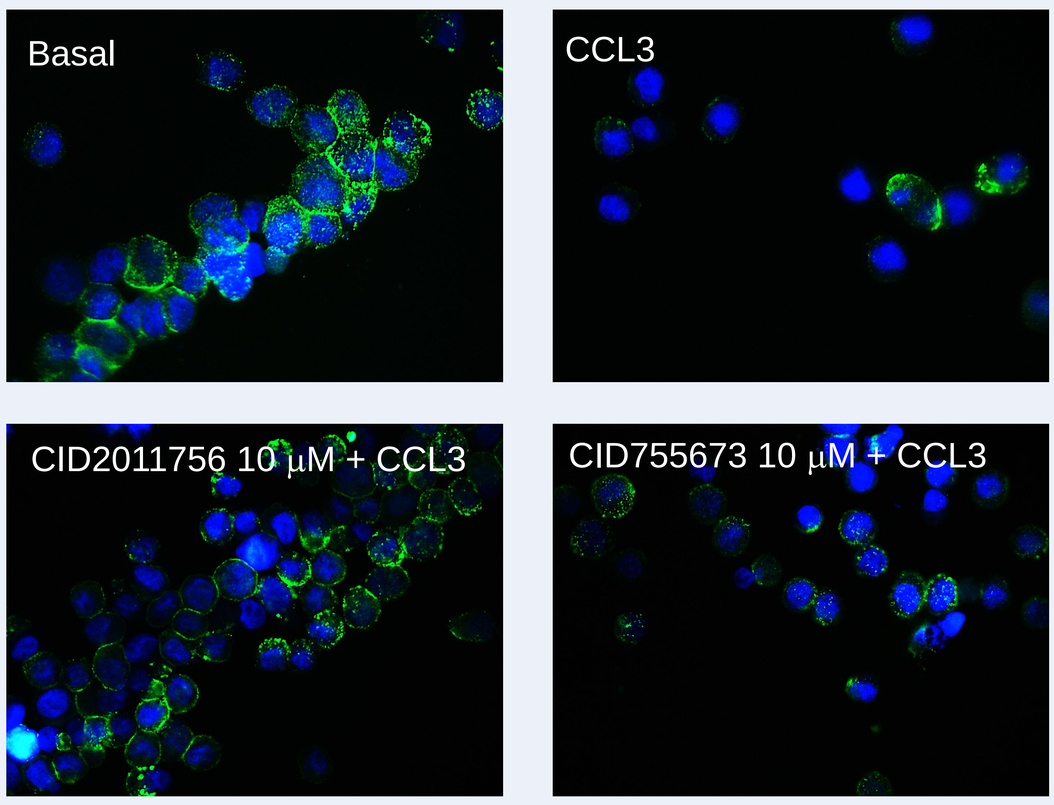

Figure 2: CCR5 receptor internalisation in THP-1 cells stimulated by CCL3 is blocked by both PKD inhibitors

CCL3-induced intracellular Ca^{2+} mobilisation is dependent of PKD in THP-1 cells, but not in MCF-7 cells.

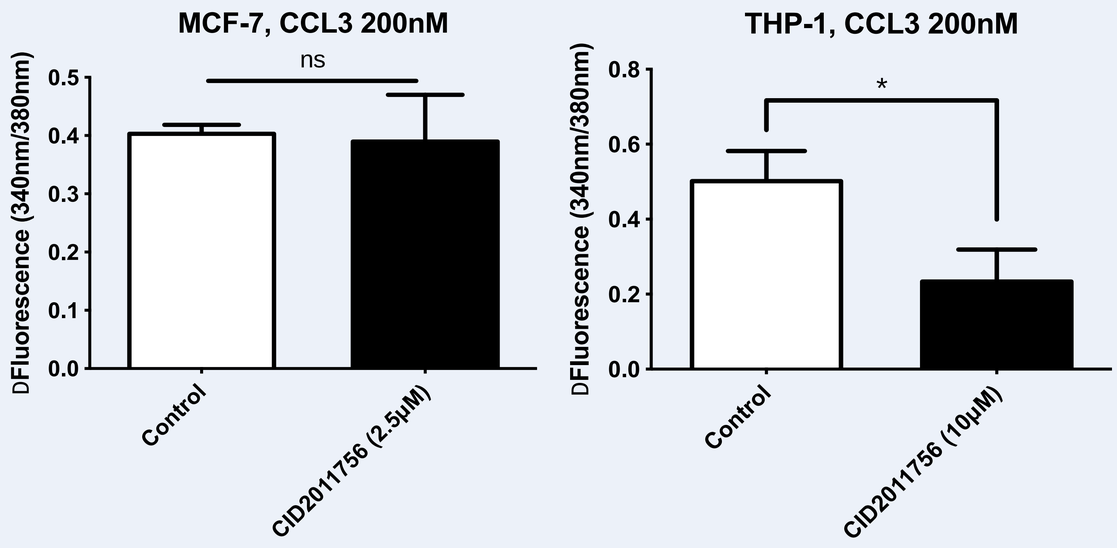

Figure 3: In the stimulation of CCL3 (200nM), Ca^{2+} release is blocked by CID2011756 in THP-1 cells, but not in MCF-7, n≥ 3

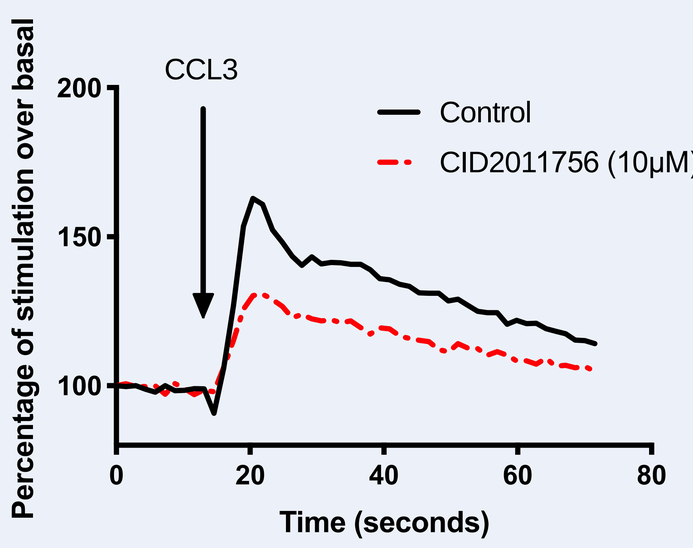

Figure 4: Representative trace of real-time intracellular calcium flux in THP-1 cells pre-treated with CID2011756 compared to untreated control

Discussion

PKD potentially plays a role in CCL3-induced CCR5 internalization (figure 1, 2). The effect of PKD varies in different cell lines (table 1) which can be explained by differentiate extent of receptor internalisation in response to chemokine stimulation and which internalisation pathway utilised. In terms of the role of PKD in CCL3-CCR5 signalling axis, intracellular calcium mobilisation is dependent of PKD in THP-1 cell but not in MCF-7 cells (figure 3). In conclusion, the effect of PKD on cellular responses can be cell type-specific.

References

1. Hanahan D, Weinberg Robert A. (2011). Cell. 144(5):646-74. 2. Balkwill F. (2004). Nature reviews Cancer. 4(7):540-50.
3. Roy A, Ye J, Deng F, Wang QJ. (2017). Biochimica et biophysica acta Reviews on cancer. 1868(1):283-94.