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Rac1 plays a role in CXCL12 but not CCL3-induced chemotaxis and Rac1 GEF inhibitor NSC23766 has off target effects on CXCR4

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ABSTRACT

Cell migration towards a chemotactic stimulus relies on the re-arrangement of the cytoskeleton, which is triggered by activation of small G proteins RhoA, Rac1 and Cdc42, and leads to formation of lamellopodia and actin polymerisation amongst other effects. Here we show that Rac1 is important for CXCR4 induced chemotaxis but not for CCR1/CCR5 induced chemotaxis. For CXCL12-induced migration via CXCR4, breast cancer MCF-7 cells are reliant on Rac1, similarly to THP-1 monocytes and Jurkat T-cells. For CCL3-induced migration via CCR1 and/or CCR5, Rac1 signalling does not regulate cell migration in either suspension or adherent cells. We have confirmed the involvement of Rac1 with the use of a specific Rac1 blocking peptide. We also used a Rac1 inhibitor EHT 1864 and a Rac1-GEF inhibitor NSC23766 to probe the importance of Rac1 in chemotaxis. Both inhibitors did not block CCL3-induced chemotaxis, but they were able to block CXCL12-induced chemotaxis. This confirms that Rac1 activation is not essential for CCL3-induced migration, however NSC23766 might have secondary effects on CXCR4. This small molecule exhibits agonistic features in internalisation and cAMP assays, whereas it acts as an antagonist for CXCR4 in migration and calcium release assays. Our findings strongly suggest that Rac1 activation is not necessary for CCL3 signalling, and reveal that NSC23766 could be a novel CXCR4 receptor ligand.

1. Introduction

Chemokines are small proteins produced by cells that can trigger cellular migration activated by G protein-coupled receptors (GPCRs), called chemokine receptors [1]. Particularly in cancer, it has been shown that these chemokine receptors play a critical role in inducing the migration of cancer cells to different parts of the body [2,3]. Several chemokine receptors are highly expressed on cancer cells, including CXCR4, CCR5 and CCR1 [4–10]. Chemokine receptors may initiate signalling through binding a ligand, a specific chemokine or a chemical. CXCL12 binds to and activates the CXCR4 and CXCR7 receptors, but it is thought that only CXCR4 activation leads to chemotaxis of cells [11]. CCL3 is a ligand for both CCR5 and CCR1; it can activate both receptors and lead to a migratory response in cells [12]. Several other ligands can also bind to either CCR1 or CCR5 or both [12,13]. In general, chemokine receptor activation leads to an activation of heterotrimeric G proteins and phosphorylation of the receptors via GRKs (G protein-coupled receptor kinases) or PKC (protein kinase c). This leads to the binding of arrestins to the phosphorylated form of the receptor and causes receptor internalisation [12,14]. Traditionally it is thought that βγ-subunits of the G proteins induce migration via activation of PI3K [15], however we have recently shown that this seems not to be the case for CCL3-induced chemotaxis in THP-1 cells [16] whereas PI3K is important for CXCL12-induced migration [5]. Both CXCL12 and CCL3-induced migration relies on the activation of Src, whereas the involvement of PKC seems to be cell type dependant [5].

Re-arrangement of the actin cytoskeleton is of major importance for chemotaxis, and the small G proteins of the rho family (rho, rac and cdc42) play important roles in this [17]. Actin filament reorganization is a dynamic process that requires both actin polymerizing and depolymerizing factors [18]. Specifically, Cdc42 and Rac1 regulate filopodia and lamellopodia formation, respectively, while RhoA regulates stress fibres and focal adhesion [19]. It has been well documented that activation of chemokine receptors leads to the occurrence of actin stress fibres and membrane ruffling [20–22]. It has also been shown that the blocking of RhoA or ROCK (rho-activated kinase) prevents migration of cells [16]. However, it is not known how chemokines and their receptors regulate the actin cytoskeleton leading to metastasis of cancer

Abbreviations: AUC, Area under Curve; EDTA, Ethylenediaminetetraacetic acid; FCS, Foetal calf serum; FSC, Forward scatter; GPCR, G protein-coupled receptor; PBS, Phosphate buffered saline; SEM, Standard Error of Means; SSC, Side scatter

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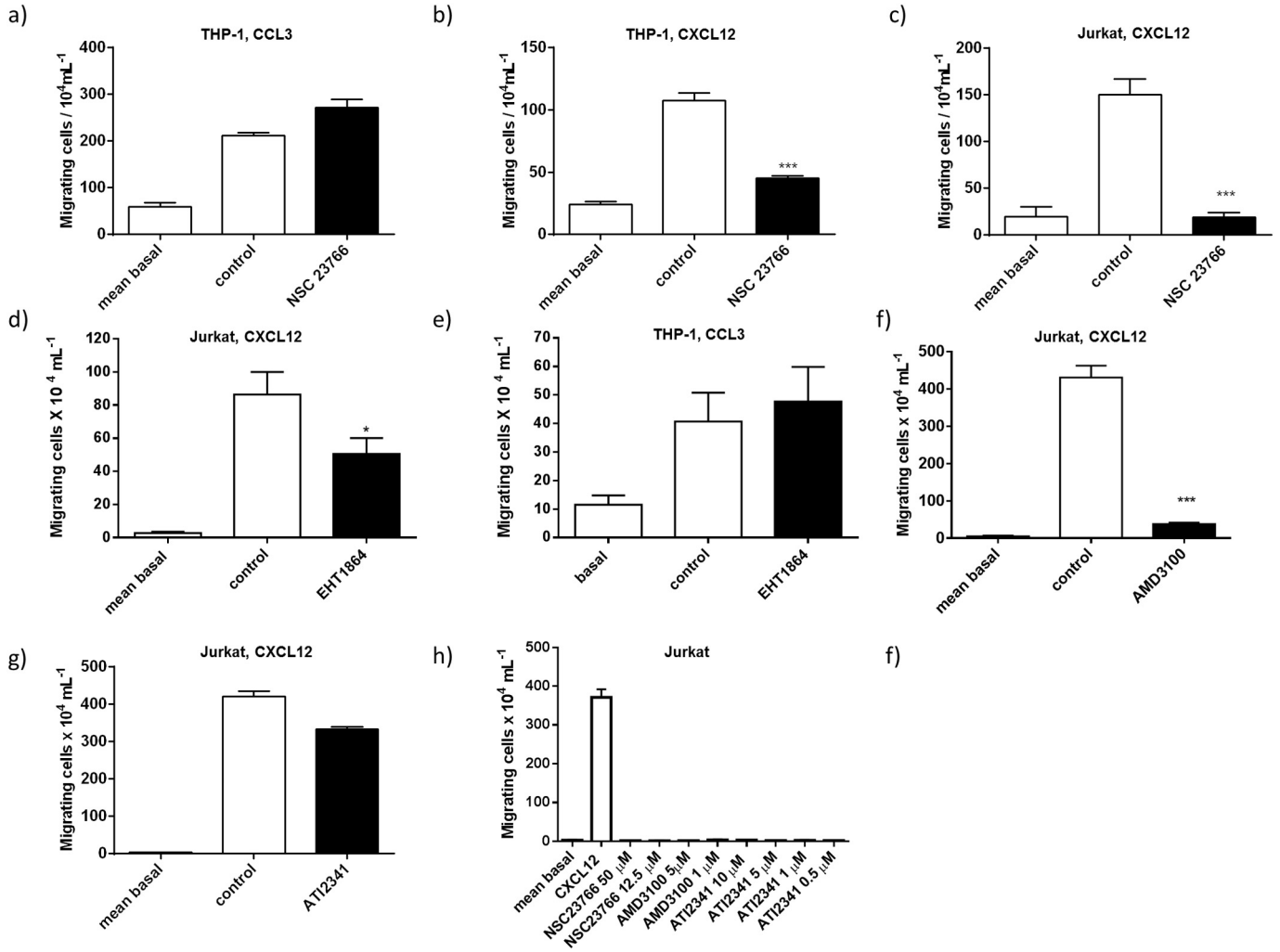

Fig. 1. NSC23766 blocks CXCL12-induced chemotaxis but not CCL3. A) Shows migratory response of THP-1 cells towards 1 nM CCL3 in untreated control cells or 100 μM NSC23766 pre-treated cells. B) Cell chemotaxis towards 1 nM CXCL12 in untreated control cells or 100 μM NSC23766 pre-treated THP-1 cells. C) Cell chemotaxis towards 1 nM CXCL12 in untreated control cells or 100 μM NSC23766 pre-treated Jurkat cells. D) Cell chemotaxis towards 1 nM CXCL12 in untreated control cells or 100 nM EHT1864 pre-treated Jurkat cells. E) Cell chemotaxis towards 1 nM CCL3 in untreated control cells or 100 nM EHT1864 pre-treated THP-1 cells. F) Cell chemotaxis towards 1 nM CXCL12 in untreated control cells or 1 μM AMD3100 pre-treated Jurkat cells, G) Cell chemotaxis towards 1 nM CXCL12 in untreated control cells or 5 μM ATI23415 pre-treated Jurkat cells. H) Cell chemotaxis towards 1 nM CXCL12 or towards NSC23766, AMD3100 or ATI23415 as chemoattractants. Data shown are the mean ± SEM of at least 3 independent experiments. (* = $p \leq 0.05$, *** = $p \leq 0.001$, One-way ANOVA with a Tukey's multiple comparisons test as post-test).

cells [23,24]. In leukocytes, chemokine receptors control activation of a small G protein, Rac1, which induces growth of actin filaments. Recent studies have shown that Rac1 is associated with CXCL12-induced chemotaxis in breast cancer cells [24], as well as modulating cell invasion and tumour metastasis in human oesophageal cancer [25]. Direct association with Rac1 also seems to affect the conformation of CXCR4 [26] and therefore might affect chemokine binding to the receptor and activation of downstream signalling partners. In this study we examined the role of Rac1 in cell chemotaxis induced by two different chemokines, CCL3 and CXCL12 respectively, in different cellular backgrounds. These results highlight the importance of characterising cell signalling networks by single receptor, and not by families, as well as considering cellular background when analysing results.

2. Materials and methods

2.1. Cells and materials

The leukemic cell line Jurkat and the monocytic cell line THP-1 were purchased from the ATCC (Teddington, UK) and both cell lines were grown as described [5]. The breast cancer cell line MCF-7 was obtained from the ATCC and grown in DMEM containing 10% FCS and 2 mM L-glutamine. The chemokine CXCL12 was obtained from Peprotech (London, UK); CCL3 has been described previously [16,27]. NSC23766, AMD3100, and H89 were purchased from Abcam (Cambridge, UK). ATI2341 was from Tocris Biosciences (Bristol, UK), EHT1864 was purchased from Cambridge Biosciences (Cambridge, UK). The anti-CXCR4 (12G5) antibody was from R & D Systems (Abingdon, UK) and the corresponding FITC labelled anti-mouse secondary antibody was from Sigma (Poole, UK). Rac1 Pull-down Activation Assay Kit was obtained from Cytoskeleton Inc. (Denver, USA), the CatchPoint cAMP Fluorescent Assay Kit was from Molecular Devices (Wokingham, UK). All other chemicals were from Fisher Scientific (Loughborough, UK).

2.2. Peptide synthesis

Two 15mer peptides, active (VDGKPVNLGLWDTAG) (W56) and inactive (VDGKPVNLGLFDTAG) (F56) were synthesized on a Multisyntech Syro I automated peptide synthesiser using standard Nα-Fmoc-based solid-phase peptide synthesis. The synthesis was carried out on a NOVA PEG Rink amide polystyrene resin (substitution: