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The role of ACKR3 in breast, lung and brain cancer

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ACKR3 atypical chemokine receptor; **bCSC** breast cancer stem cells; **CNS** central nervous system; **CXCL12** C-X-C motif chemokine 12; **CXCL11** C-X-C motif chemokine 11; **CXCR4** C-X-C chemokine receptor type 4; **EGF** epidermal growth factor; **EGFR** epidermal growth factor receptor; **ER** oestrogen receptor; **ERK** extracellular-signal regulated kinases; **GPCR** G protein-coupled receptor; **IDH1/IDH2** isocitrate dehydrogenase 1 and 2; **mLLC** mouse Lewis lung carcinoma; **MMP** matrix metalloproteinase; **NSCLC** non-small cell lung cancer; **STAT3** signal transducer and activator of transcription 3; **VCAM-1** vascular cell-adhesion molecule-1; **VEGF** Vascular endothelial growth factor; **TGF-β1** Transforming Growth Factor-beta 1; **XIST** X-inactive-specific transcript.

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Abstract

Recent reports regarding the significance of chemokine receptors in disease have put a spotlight on ACKR3. This atypical chemokine receptor is overexpressed in numerous cancer types and has been involved in the modulation of tumour cell proliferation and migration, tumour angiogenesis or resistance to drugs, thus contributing to cancer progression and metastasis occurrence. Here we focus on the clinical significance and potential mechanisms underlying the pathological role of ACKR3 in breast, lung and brain cancer and discuss its possible relevance as prognostic factor and potential therapeutic target in these contexts.

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Introduction

ACKR3 (atypical chemokine receptor 3, also known as CXCR7) is a seven transmembrane domain atypical chemokine receptor that belongs to the class A of G protein-coupled receptors (GPCRs). Both C-X-C motif chemokine 12 (CXCL12) and C-X-C motif chemokine 11 (CXCL11) bind to the receptor. CXCL12 also binds to CXCR4 (C-X-C chemokine receptor type 4), and likewise CXCL11 binds to CXCR3 (C-X-C chemokine receptor type 3), another chemokine receptor of this family. Since the first description of ACKR3 as a co-receptor for HIV entry (Shimizu *et al.*, 2000), it has become clear that this receptor has a prominent role in many pathological contexts. ACKR3 expression was found to be up-regulated in a variety of pathological conditions related to inflammation, infection, or ischemia. For instance, ACKR3 expression is increased in inflammatory bowel disease, encephalitis, rheumatoid arthritis, acute renal failure, Epstein-Barr virus type I infection, or after permanent middle cerebral artery occlusion (Sánchez-Martín *et al.*, 2013). Importantly, altered ACKR3 expression patterns have also been detected in numerous cancer types, such as prostate, kidney, liver, cervix, brain, lung and breast cancer (reviewed in Sánchez-Martín *et al.*, 2013; Freitas *et al.*, 2014). In this review, we will focus on the evidence linking altered ACKR3 expression with the progression of breast, lung and glioma tumours and discuss the underlying molecular mechanisms.

ACKR3 in Breast Cancer

Breast cancer is the most prevalent cancer among women worldwide (Siegel *et al.*, 2016). Several studies describe an association between the CXCL12-ACKR3 axis and disease progression and poor overall survival of breast cancer patients. As a result, interest in this receptor is markedly increasing, as it may be a potential therapeutic target. Previous reports indicate that ACKR3 expression in breast cancer patient samples (*in situ* and invasive lobular carcinomas) is much higher when compared to adjacent tissues and normal mammary

epithelium. This was verified at protein and mRNA levels (Miao *et al.*, 2007; Wani *et al.*, 2014; Li *et al.*, 2015; Behnam Azad *et al.*, 2016). High expression was also detected in metastatic tissues (Wu *et al.*, 2016; PF Yu *et al.*, 2017). Notably, ACKR3 overexpression correlates with poor prognosis and lung metastasis-free survival (Behnam Azad *et al.*, 2016; Wu *et al.*, 2016; PF Yu *et al.*, 2017).

Furthermore, there is an association between higher levels of ACKR3 and worse outcomes in terms of disease-free survival (Hassan *et al.*, 2009), and a positive correlation between ACKR3 expression level and lymph node metastasis: ACKR3 expression is higher in primary tumours of patients with lymphatic metastasis than in those of metastasis-free patients (Li *et al.*, 2015). Taken together, these findings indicate that ACKR3 plays a role in breast cancer progression and metastasis occurrence.

Several *in vitro* and *in vivo* studies have found that higher levels of ACKR3 result in increased cell proliferation and invasive migration, promoting tumour growth and metastasis (Miao *et al.*, 2007; Xue *et al.*, 2013; Gao *et al.*, 2015; Li *et al.*, 2015). However, the molecular mechanisms linking enhanced ACKR3 expression to these processes are not fully elucidated. Research performed in model cell lines with ectopically over-expressed ACKR3 and CXCR4 receptors or in different cell types with endogenous levels of these proteins have put forward several potential mechanisms. In mouse migrating interneurons, ACKR3 has been shown to be key for migration by scavenging CXCL12, thus preventing co-expressed CXCR4 from desensitization and degradation and allowing adequate levels of CXCL12-CXCR4 signalling (Sánchez-Alcañiz *et al.*, 2011; Abe *et al.*, 2014). These data suggest that high ACKR3 expression in breast cancer would foster CXCR4-dependent cascades. Such a scavenging role is related to constitutive and ligand-dependent ACKR3 internalization and recycling observed in different cell types (Naumann *et al.*, 2010), including breast cancer

cells (Luker *et al.*, 2010). This allows receptor trafficking to lysosomes and degradation of receptor-bound CXCL12 (Luker *et al.*, 2010; Naumann *et al.*, 2010). Consistent with this notion, uptake of CXCL12 by ACKR3-positive (ACKR3+) breast cancer cells increases proliferation and metastatic potential of CXCR4+ cells (Luker *et al.*, 2012). Interestingly, ACKR3 ligands trigger β-arrestin recruitment to the receptor in model cell systems (Luker *et al.*, 2009; Rajagopal *et al.*, 2010; Benredjem *et al.*, 2017) and it has been reported that ACKR3 internalization and the resulting chemokine degradation are dependent on β-arrestin in breast cancer cells (Luker *et al.*, 2010). However, constitutive receptor internalization and recycling can also take place in a ligand-independent way. Recent data show that β-arrestins are dispensable for chemokine scavenging (Montpas *et al.*, 2018), suggesting that other ACKR3-interacting proteins might be involved in the internalization process, depending on the experimental conditions and cellular context.

In addition to its ligand scavenging properties, it cannot be ruled out that the interaction of ACKR3 with β-arrestins (or other interacting proteins to be identified) may also trigger other downstream cascades in a cell-type specific way (Sánchez-Alcañiz *et al.*, 2011). ACKR3 recruitment of β-arrestins might also modify CXCR4 signalling, by recruiting β-arrestin2 away from co-expressed CXCR4 (Coggins *et al.*, 2014), or via ACKR3/CXCR4 co-internalization, as reported in MCF7 breast cancer cells (Sánchez-Martín *et al.*, 2013).

Therefore, it is tempting to suggest that both CXCR4 and ACKR3 cooperate to promote tumour growth and metastasis through different interaction and crosstalk mechanisms (Sierro *et al.*, 2007; Levoye *et al.*, 2009; Luker *et al.*, 2009, 2010). While some reports indicate that CXCR4 is overexpressed in breast cancer patient samples, with both receptors co-localized (Inaguma *et al.*, 2015), others have shown that CXCR4 and ACKR3 are predominantly expressed on separated populations of breast cancer cells (Luker *et al.*, 2012). Additionally, CXCR4 and ACKR3 co-expression in the MTLn3 breast cancer cell line decreases invasion in response to CXCL12 (Hernandez *et al.*, 2011). Given the discrepancies in the available data, it is key to further investigate whether CXCR4 and ACKR3 are expressed in the same

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or distinct subpopulations of breast cancer cells to better understand these potential crosstalk mechanisms.

In addition to CXCR4, ACKR3 may also functionally interact with other key signalling cascades in breast cancer, such as estrogen and EGF receptors. Analysis of luminal type breast cancer tumour samples revealed a positive correlation between ACKR3 and estrogen receptor (ER) levels.. Overexpression of ACKR3 can result in increased estrogen signalling in ER+ breast tumour cells, and is closely related to tamoxifen insensitivity, which would make ACKR3 a relevant target specifically in ER-positive breast cancer (Hao *et al.*, 2018).

ACKR3 and Epidermal Growth Factor Receptor (EGFR) were also found co-localized in ER+ breast cancer tissues. *In vitro* studies show that EGFR expression correlates with ACKR3 levels in breast cancer cell lines and, interestingly, that EGFR is absent in cells lacking ACKR3. Furthermore, partial depletion of ACKR3 in a breast cancer cell line results in decreased proliferation in response to Epidermal Growth Factor (EGF) and decreased activation of the Extracellular-signal Regulated Kinases (ERK) pathway and level of EGFR phosphorylation. These findings strongly suggest a cooperation between both receptors in tumour proliferation (Salazar *et al.*, 2014).

In addition to the aforementioned roles involving other receptors, ACKR3 has an independent effect on cell cycle or apoptosis-related cascades in breast cancer cells. Decreasing ACKR3 levels alters expression of cell cycle related proteins, and leads to cell cycle arrest, hence reducing cell proliferation (Salazar *et al.*, 2014). Additionally, knock-down of the receptor in the MCF7 breast cancer cell line increased the expression of the pro-apoptotic caspase 3 and 8 proteins (Gao *et al.*, 2015). In addition, ACKR3 expression enhances breast cancer proliferation through Signal transducer and activator of transcription 3 (STAT3) activation,

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which has previously been linked to apoptosis resistance and cell cycle progression in invasive breast cancer (Wani *et al.*, 2014; Li *et al.*, 2015).

Another key event in breast cancer is cell migration and invasion, ultimately leading to metastasis occurrence, which is the primary cause of death of the majority of breast cancer patients. Migration and invasion events require degradation of basement membrane extracellular matrix proteins by matrix metalloproteinases (MMPs). ACKR3 expression is associated with the secretion of MMP2, MMP3 and MMP9 (Zabel *et al.*, 2010; Gao *et al.*, 2015). ACKR3 overexpression also accompanies mesenchymal to epithelial transition, a pathway leading to metastatic events (Leontovich *et al.*, 2012).

In addition to metastasis, a current challenge for breast cancer targeted therapies are breast cancer stem cells (bCSCs), a population of cells located within the tumour with high proliferative capacity and exhibiting resistance to therapeutics. It was demonstrated that ACKR3 is crucial for tumourigenicity and maintenance of stem cell properties of bCSCs (Tang *et al.*, 2016). Given the role of this receptor in both metastasis and bCSCs within the primary tumour, it appears to be an attractive target for new therapeutic approaches for breast cancer.

Accumulating evidence suggests that ACKR3 also plays a pivotal role in tumour-associated vasculature. One study assessing ACKR3 expression in tumour samples (Behnam Azad *et al.*, 2016) showed an intense vascular staining specifically in the endothelium of tumour-associated blood vessels. In contrast, the receptor was not expressed in healthy tissue blood vessels. Another report demonstrated that concomitant expression of ACKR3 in tumour and tumour-associated vasculature promotes cell-cell interaction (Miao *et al.*, 2007), suggesting a role for this receptor in modulating cell adhesion in the tumour microenvironment. ACKR3

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also enhances vascular cell-adhesion molecule-1 (VCAM-1) expression (Wani *et al.*, 2014). Since tumour cell adhesion to the basal membrane is crucial for the invasive process, these data suggest that ACKR3 mediates this step. Furthermore, a recent study showed that ACKR3 down-regulation decreases Vascular endothelial growth factor (VEGF) production and tube formation (Qian *et al.*, 2018), suggesting a role for this receptor in angiogenesis, an important event that leads to tumour cell survival and migration. Nevertheless, another report using a different experimental model suggested that vascular ACKR3 suppresses tumour proliferation and decreases metastatic potential of tumour cells (Stacer *et al.*, 2015). These discrepancies may be explained by the different models used in each study or might suggest a context-dependent role for ACKR3, that requires further investigation. On the other hand, it is worth noting that ACKR3 also increases tumour-promoting macrophages and recruitment to the tumour site (Wani *et al.*, 2014), a key event in determining the tumour microenvironment.

In summary, these studies point to a pivotal role for ACKR3 in breast cancer progression and metastasis by promoting survival, proliferation and migration of malignant cells. Moreover ACKR3 modulates angiogenic pathways and helps to shape the tumour microenvironment, thus further controlling tumour growth (Fig.1). Although more studies are needed to clarify the exact mechanisms by which ACKR3 operates, accumulating evidence strongly indicates that ACKR3 is a potential therapeutic target in breast cancer.

ACKR3 in lung cancer

Lung cancer is the most frequent cause of cancer-related death in men and the second most frequent in women. Initial studies on ACKR3 reported an increased expression of ACKR3 in malignant lung carcinoma biopsy material when compared to healthy human lung samples (Burns *et al.*, 2006). Further analysis of ACKR3 expression in multiple cancer types, including lung squamous cell carcinoma, showed localised ACKR3 staining on the tumour

vasculature. These first findings suggested a role for ACKR3 in lung cancer (Miao *et al.*, 2007), that was further investigated *in vivo* using mice engrafted with mouse Lewis lung carcinoma (mLLC) cells or human lung carcinoma cells. These studies demonstrated that both diminishing ACKR3 expression and inhibiting ACKR3 signalling by the ACKR3 ligand CCX754 resulted in decreased tumour growth. Interestingly, the extent to which CCX754 decreased tumour growth was almost similar to the marketed chemotherapy drug melphalan, showing the potential of targeting ACKR3 in lung cancer (Burns *et al.*, 2006; Miao *et al.*, 2007).

ACKR3 expression is found in many types of lung cancer. Investigation of human biopsy material indicates expression in small cell lung cancer (Imai *et al.*, 2010), and the non-small cell lung cancer (NSCLC) subtypes adenocarcinoma, squamous cell carcinoma and large cell carcinoma (Miao *et al.*, 2007; Goldmann *et al.*, 2008; Iwakiri *et al.*, 2009; Imai *et al.*, 2010; Franco *et al.*, 2012).

Within the Human Protein Atlas program, a recent initiative to define the oncological proteome, mRNA expression levels of ACKR3 were investigated in 794 lung cancer biopsies (Uhlen *et al.*, 2017). Comparison of the expression of ACKR3 with overall survival rate of patients showed an unfavourable outcome for high ACKR3 expression. This trend did not have the statistical power to be used for prognostic purposes, but it highlights the relevance of ACKR3 expression in lung cancer (Uhlen *et al.*, 2017).

Surgical removal of lung tumours is one of the standard treatment procedures for lung cancer. However, tumour recurrence is often observed and is related to poor prognosis (Pearson, 1999). A study of ACKR3 mRNA expression in the surgical specimens from 79 patients who underwent complete resection showed that ACKR3 mRNA expression could be

used as a postoperative 5-year disease-free prognostic marker in stage I NSCLC patients Furthermore, there was a significantly higher ACKR3 expression in pathological stage I NSCLC patients with postoperative recurrence of secondary tumours, compared to recurrence-free patients (Iwakiri *et al.*, 2009). These results suggest that ACKR3 may be related to the process by which NSCLC form secondary tumours.

A factor that has been linked to metastasis and thus the formation of secondary tumours of lung cancer is Transforming Growth Factor beta 1 (TGF-β1) (Massagué, 2008). The mRNA and protein expression of ACKR3 is regulated by TGF-β1, and the combination of high TGF-β1 and ACKR3 expression correlates with poor patient survival (Wu *et al.*, 2016). TGF-β1-mediated functional effects, including cancer cell motility and epithelial-to-mesenchymal transition, appear to depend on ACKR3 (Massagué, 2008; Wu *et al.*, 2016). Furthermore, epithelial-to-mesenchymal transition of cancer cells leads to the formation of cancer stem cells which show high metastatic properties (Singh and Settleman, 2010) and TGF-β1 induces cancer stem cell formation in a ACKR3-dependent manner (Wu *et al.*, 2016). Collectively, these findings suggest a key role of ACKR3 in cancer stem cell formation, what might underlie ACKR3-mediated formation of secondary tumours.

In lung cancer, mutations in the EGFR, KRAS and ALK genes are mutually exclusive and can be used to define responsiveness to different chemotherapies. A study on the presence of EGFR mutations showed that ACKR3 mRNA expression is higher in lung cancer patients with mutated EGFR compared to wild type EGFR patients, suggesting a role for ACKR3 in EGFR-mediated lung cancer (Iwakiri *et al.*, 2009). Furthermore, an *in vivo* study in which Lkb1 and Pten were conditionally knocked down in mice showed that this leads to development of the lung cancer subtype, squamous cell carcinoma (Xu *et al.*, 2014). Transcriptomics data of the Lkb1(-/-)/Pten(-/-) squamous cell carcinoma tumour showed an increase in ACKR3 expression when compared to healthy lung tissue, suggesting a role of

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ACKR3 in Lkb1/Pten-mediated lung cancer (Xu *et al.*, 2014). Altogether, these observations suggest a universal role of ACKR3 in multiple lung cancer subtypes.

ACKR3 in Glioma

The term glioma encloses a variety of intrinsic central nervous system tumours. These tumours were initially classified based on their presumed cellular origin (astrocytoma, oligodendroglioma, oligoastrocytoma) and extension of infiltration (diffuse or non-diffuse glioma). Regardless of the sub-classification, glioma are divided into three malignancy grades (II, III and IV) considering their mitotic activity, necrosis and florid microvascular proliferation. Glioblastoma is the most malignant one (grade IV) and is categorised as either 'secondary' or 'primary' depending on whether or not there is evidence of a progression from a lower grade glioma. However, in 2016, the World Health Organization (WHO) introduced a genotypic classification based on the presence of recurrent point mutations in isocitrate dehydrogenase 1 and 2 (*IDH1/IDH2*) genes, dividing glioblastoma into glioblastoma-IDH-wildtype and glioblastoma-IDH-mutant. Interestingly, the majority of secondary glioblastoma are IDH-mutants whereas primary glioblastoma are typically IDH-wildtype (Parsons *et al.*, 2008). Recently, it was proposed that ACKR3 influences prognosis in human glioma depending on the IDH classification (Birner *et al.*, 2015). In fact, ACKR3 expression in tumour-associated vessels predicts a better prognosis in IDH1-wildtype glioma whereas it has opposite consequences in the IDH1-mutant subtype. In addition, mRNA as well as protein levels of ACKR3 were found to be up-regulated in glioma (Calatozzolo *et al.*, 2011) and to positively correlate with WHO grade in several studies (Hattermann *et al.*, 2010; Bianco *et al.*, 2014; Walters *et al.*, 2014; Birner *et al.*, 2015). ACKR3 localisation also changes with WHO grades. In fact, in grade II glioma, ACKR3 is mainly expressed in cancer cells. In grade III, it is present primarily in tumour vascular endothelial cells and only marginally in cancer cells. In glioblastoma, ACKR3 is found in cancer cells, in pseudo-

palisades nearest to necrotic areas and in the tumour endothelium (Bianco *et al.*, 2014). Standard care for the treatment of WHO grade III and IV glioma consists of surgery followed by chemotherapy (temozolomide) that can be combined with intermediate-frequency alternating electric fields (Touat *et al.*, 2017). However, glioma exhibit both radio (Han *et al.*, 2017) and drug resistance (Stavrovskaya *et al.*, 2016), making the current therapies ineffective with a median survival ranging from 12 to 16 months after diagnosis (Gilbert *et al.*, 2014). Accumulating evidence suggests that ACKR3 might play a role in these acquired resistances. In fact, CXCL12 reverses the anti-proliferative effect of non-toxic concentrations of temozolomide in C6 rat cells isolated from murine glioma (Hattermann *et al.*, 2012). Accordingly, CXCL12 also reduces temozolomide-induced apoptosis. These CXCL12 effects might involve ACKR3, which is expressed at much higher level than CXCR4 in C6 cells. Corroborating these findings, the presence of the ACKR3 modulator CCX771 after irradiation provokes tumour regression in nude mice injected with U251 glioma cells (Walters *et al.*, 2014). In addition, treatment with the ACKR3 ligand CCX662 (able to induce β-arrestin recruitment to ACKR3 and inhibit CXCL12-dependent migration) extends the survival of rats with nitrosourea-induced brain tumours after irradiation. However, in a more aggressive model consisting in the injection of C6 glioma cells in rats, only the combination of irradiation with CCX662 treatment extends survival.

Contradictory results are emerging regarding the role of ACKR3 in glioma cell proliferation. In fact, in the U373 glioblastoma cell line and in human foetal astrocytes, CXCL12-induced proliferation is prevented by the ACKR3 ligand CCX733 (Calatozzolo *et al.*, 2011). On the other hand, CXCL12 does not influence either proliferation or migration of A764 and U343 glioma cells (Hattermann *et al.*, 2010). Moreover, in co-cultures of U87 cells and human brain microvascular endothelial cells, the receptor was found to have no trophic effect (Rao *et al.*, 2012). Long non-coding RNA X-inactive-specific transcript (XIST) is up-regulated in glioma endothelial cells forming the blood-tumour barrier (H Yu *et al.*, 2017) and increases glioma angiogenesis by indirectly controlling ACKR3 expression. In fact the study showed that

down-regulation of XIST increases miRNA-137 expression, which in turns inhibits the expression of forkhead box C1 (FOXC1), a transcription factor that promotes ACKR3 expression.

The common view of ACKR3 as an atypical chemokine receptor unable to activate G proteins (Naumann *et al.*, 2010) was challenged in 2012 when it was suggested that the receptor can signal through $G_{i/o}$ proteins in astrocytes and human glioma cells (Odemis *et al.*, 2010). This study showed that CXCL12 induces activation of G proteins and Ca^{2+} influx in rat astrocytes in primary culture. Both effects persisted in cultures obtained from CXCR4 knockout mice and were abolished by pre-treatment of cells with an ACKR3 small interfering RNA. Moreover, both CXCL12-induced proliferation and migration of rat astrocytes did not require CXCR4 expression and were inhibited by *Pertussis toxin,* suggesting that ACKR3 influences migration and proliferation of astrocytes in a G protein-dependent fashion. On the other hand, in the same study, CXCL11 did not activate G proteins suggesting that the ability of ACKR3 to activate G proteins in astrocytes depends on the nature of the agonist. The ability of ACKR3 to activate G proteins has so far only been found in glial cells, but not in other cell types (Levoye *et al.*, 2009; Rajagopal *et al.*, 2010; Kumar *et al.*, 2012). This unique capability of glial ACKR3 to activate G proteins certainly warrants further exploration providing that it is confirmed in independent studies. In another study, CXCL12 also triggered MAPK phosphorylation in glioma cells *via* phospholipase C but not AKT and p38 (Hattermann *et al.*, 2010). Whether this effect requires ACKR3 coupling to G proteins has not been investigated.

Collectively, previously published data show a high ACKR3 expression in glioma and glioma cell lines. Although its role in the progression of this cancer type and the underlying signalling mechanisms need to be fully characterised, available data indicate that this receptor plays an important role in glioma drug/radio resistance and angiogenesis.

Conclusion

Extended literature analysis points to a pivotal role of ACKR3 in breast, lung and brain cancers. Expression of this atypical chemokine receptor, both at mRNA and protein levels, is higher in tumour samples, when compared to healthy tissues. Moreover, increasing evidence indicates that receptor expression positively correlates with poor prognosis, disease grade and reoccurrence, making it a valuable biomarker for diagnosis and prognosis in certain breast, lung and brain cancer types. ACKR3 is also emerging as a key player of cancer progression and metastasis occurrence, *via* actions on both tumour cells, favouring cell proliferation and invasion, and the tumour microenvironment by modulating angiogenesis (Fig 1). The use of relevant animal or cellular models with endogenous levels of chemokine receptors will allow a better knowledge of the molecular mechanisms involved, the stimuli leading to ACKR3 overexpression in cancer contexts and the crosstalk of ACKR3 with key oncogenic signalling cascades. Insight into these mechanisms is essential to help develop new strategies to limit tumour progression and avoid resistance to therapy.

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References

Abe P, Mueller W, Schütz D, MacKay F, Thelen M, Zhang P, and Stumm R (2014) CXCR7 prevents excessive CXCL12-mediated downregulation of CXCR4 in migrating cortical interneurons. *Development* **141**:1857–1863.

Behnam Azad B, Lisok A, Chatterjee S, Poirier JT, Pullambhatla M, Luker GD, Pomper MG, and Nimmagadda S (2016) Targeted Imaging of the Atypical Chemokine Receptor 3 (ACKR3/CXCR7) in Human Cancer Xenografts. *J Nucl Med* **57**:981–988.

Benredjem B, Girard M, Rhainds D, St-Onge G, and Heveker N (2017) Mutational analysis of atypical chemokine receptor 3 (ACKR3/CXCR7) interaction with its chemokine ligands CXCL11 and CXCL12. *J Biol Chem* **292**:31–42.

Bianco AM, Uno M, Oba-Shinjo SM, Clara CA, de Almeida Galatro TF, Rosemberg S, Teixeira MJ, and Nagahashi Marie SK (2014) CXCR7 and CXCR4 Expressions in Infiltrative Astrocytomas and Their Interactions with HIF1α Expression and IDH1 Mutation. *Pathol Oncol Res* **21**:229–240.

Birner P, Tchorbanov A, Natchev S, Tuettenberg J, and Guentchev M (2015) The chemokine receptor CXCR7 influences prognosis in human glioma in an IDH1-dependent manner. *J Clin Pathol* **68**:830–4.

Burns JM, Summers BC, Wang Y, Melikian A, Berahovich R, Miao Z, Penfold MET, Sunshine MJ, Littman DR, Kuo CJ, Wei K, Mcmaster BE, Wright K, Howard MC, and Schall TJ (2006) A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival , cell adhesion , and tumor development. *J Exp Med* **203**:2201–2213.

Calatozzolo C, Canazza A, Pollo B, Di Pierro E, Ciusani E, Maderna E, Salce E, Sponza V, Frigerio S, Di Meco F, Schinelli S, and Salmaggi A (2011) Expression of the new CXCL12 receptor, CXCR7, in gliomas. *Cancer Biol Ther* **11**:242–253.

Coggins NL, Trakimas D, Chang SL, Ehrlich A, Ray P, Luker KE, Linderman JJ, and Luker GD (2014) CXCR7 controls competition for recruitment of β-arrestin 2 in cells expressing both CXCR4 and CXCR7. *PLoS One* **9**:1–14.

Franco R, Pirozzi G, Scala S, Cantile M, and Scognamiglio G (2012) CXCL12-binding receptors expression in non-small cell lung cancer relates to tumoral microvascular density and CXCR4 positive circulating tumoral cells in lung draining venous blood. *Eur J Cardio-Thoracic Surg* **41**:368–375.

Freitas C, Desnoyer A, Meuris F, Bachelerie F, Balabanian K, and Machelon V (2014) The relevance of the chemokine receptor ACKR3/CXCR7 on CXCL12-mediated effects in cancers with a focus

on virus-related cancers. *Cytokine Growth Factor Rev* **25**:307–316.

Gao W, Mei X, Wang J, Zhang X, and Yuan Y (2015) ShRNA-mediated knock-down of CXCR7 increases TRAIL-sensitivity in MCF-7 breast cancer cells. *Tumor Biol* **36**:7243–7250.

Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, Colman H, Chakravarti A, Pugh S, Won M, Jeraj R, Brown PD, Jaeckle KA, Schiff D, Stieber VW, Brachman DG, Werner-Wasik M, Tremont-Lukats IW, Sulman EP, Aldape KD, Curran WJ, and Mehta MP (2014) A Randomized Trial of Bevacizumab for Newly Diagnosed Glioblastoma. *N Engl J Med* **370**:699–708.

Goldmann T, Drömann D, Radtke J, Marwitz S, S. Lang D, Schultz H, and Vollmer E (2008) CXCR7 transcription in human non-small cell lung cancer and tumor-free lung tissues; possible regulation upon chemotherapy. *Virchows Arch* **452**:347–348.

Han X, Xue X, Zhou H, and Zhang G (2017) A molecular view of the radioresistance of gliomas. *Oncotarget* **8**:100931–100941.

Hao M, Weng X, Wang Y, Sun X, Yan T, Li Y, Hou L, Meng X, and Wang J (2018) Targeting CXCR7 improves the efficacy of breast cancer patients with tamoxifen therapy. *Biochem Pharmacol* **147**:128–140.

Hassan S, Ferrario C, Saragovi U, Quenneville L, Gaboury L, Baccarelli A, Salvucci O, and Basik M (2009) The influence of tumor-host interactions in the stromal cell-derived factor-1/CXCR4 ligand/receptor axis in determining metastatic risk in breast cancer. *Am J Pathol* **175**:66–73, American Society for Investigative Pathology.

Hattermann K, Held-feindt J, Lucius R, Müerköster SS, Penfold MET, Schall TJ, and Mentlein R (2010) The Chemokine Receptor CXCR7 Is Highly Expressed in Human Glioma Cells and Mediates Antiapoptotic Effects. 3299–3309.

Hattermann K, Mentlein R, and Held-Feindt J (2012) CXCL12 mediates apoptosis resistance in rat C6 glioma cells. *Oncol Rep* **27**:1348–1352.

Hernandez L, Magalhaes MAO, Coniglio SJ, Condeelis JS, and Segall JE (2011) Opposing roles of CXCR4 and CXCR7 in breast cancer metastasis. *Breast Cancer Res* **13**:19–21.

Hoffmann F, Müller W, Schütz D, Penfold ME, Wong YH, Schulz S, and Stumm R (2012) Rapid uptake and degradation of CXCL12 depend on CXCR7 carboxyl-terminal serine/threonine residues. *J Biol Chem* **287**:28362–28377.

Imai H, Sunaga N, Shimizu Y, Kakegawa S, Shimizu KI, San T, Ishizuka T, Oyama T, Sait R, Minna JD, and Marl M (2010) Clinicopathological and Therapeutic Significance of CXCL12 Expression in Lung Cancer. *Int J Immunopathol Pharmacol* **23**:153–164.

Inaguma S, Riku M, Ito H, Tsunoda T, Ikeda H, and Kasai K (2015) GLI1 orchestrates CXCR4/CXCR7 signaling to enhance migration and metastasis of breast cancer cells. *Oncotarget* **6**.

Iwakiri S, Mino N, Takahashi T, Sonobe M, Nagai S, Okubo K, Wada H, Date H, and Miyahara R (2009) Higher Expression of Chemokine Receptor CXCR7 Is Linked to Early and Metastatic Recurrence in Pathological Stage I Nonsmall Cell Lung Cancer. *Cancer* 2580–2593.

Kumar R, Tripathi V, Ahmad M, Nath N, Mir RA, Chauhan SS, and Luthra K (2012) CXCR7 mediated Giα independent activation of ERK and Akt promotes cell survival and chemotaxis in T cells. *Cell Immunol* **272**:230–241, Elsevier Inc.

Leontovich AA, Zhang S, Quatraro C, Iankov I, Veroux PF, Gambino MW, Degnim A, McCubrey J, Ingle J, Galanis E, and D'Assoro AB (2012) Raf-1 oncogenic signaling is linked to activation of mesenchymal to epithelial transition pathway in metastatic breast cancer cells. *Int J Oncol* **40**:1858–1864.

Levoye A, Balabanian K, Baleux F, Bachelerie F, and Lagane B (2009) CXCR7 heterodimerizes with CXCR4 and regulates CXCL12-mediated G protein signaling. *Blood* **113**:6085–6093.

Li J-T, Jia L-T, Liu N-N, Zhu X-S, Liu Q-Q, Wang X-L, Yu F, Liu Y-L, Yang A-G, and Gao C-F (2015) MiRNA-101 inhibits breast cancer growth and metastasis by targeting CX chemokine receptor 7. *Oncotarget* **6**:30818–30830.

Luker, Gupta M, and Luker GD (2009) Imaging chemokine receptor dimerization with firefly luciferase complementation. *FASEB J* **23**:823–834.

Luker KE, Gupta M, Steele JM, Foerster BR, and Luker GD (2009) Imaging Ligand-Dependent Activation of CXCR7. *Neoplasia* **11**:1022–1035.

Luker KE, Lewin SA, Mihalko LA, Schmidt BT, Winkler JS, Coggins NL, Thomas DG, and Luker GD (2012) Scavenging of CXCL12 by CXCR7 promotes tumor growth and metastasis of CXCR4-positive breast cancer cells. *Oncogene* **31**:4750–4758, Nature Publishing Group.

Luker KE, Steele JM, Mihalko LA, Ray P, and Gary D (2010) Constitutive and Chemokine-dependent Internalization and Recycling of CXCR7 in Breast Cancer Cells to Degrade Chemokine Ligands. *Oncogene* **29**:4599–4610.

Luker, Steele JM, Mihalko LA, Ray P, and Luker GD (2010) Constitutive and chemokine-dependent internalization and recycling of CXCR7 in breast cancer cells to degrade chemokine ligands. *Oncogene* **29**:4599–4610, Nature Publishing Group.

Massagué J (2008) TGF β in Cancer. *Cell* **134**:215–230.

Miao Z, Luker KE, Summers BC, Berahovich R, Bhojani MS, Rehemtulla A, Kleer CG, Essner JJ, Nasevicius A, Luker GD, Howard MC, and Schall TJ (2007) CXCR7 (RDC1) promotes breast and lung tumor growth in vivo and is expressed on tumor-associated vasculature. *Proc Natl Acad Sci U S A* **104**:15735–40.

Montpas N, St-Onge G, Nama N, Rhainds D, Benredjem B, Girard M, Hickson G, Pons V, and Heveker N (2018) Ligand-specific conformational transitions and intracellular transport are required for atypical chemokine receptor 3–mediated chemokine scavenging. *J Biol Chem* **293**:893–905.

Naumann U, Cameroni E, Pruenster M, Mahabaleshwar H, Raz E, Zerwes HG, Rot A, and Thelen M (2010) CXCR7 functions as a scavenger for CXCL12 and CXCL11. *PLoS One* **5**.

Odemis V, Boosmann K, Heinen A, Küry P, and Engele J (2010) CXCR7 is an active component of SDF-1 signalling in astrocytes and Schwann cells. *J Cell Sci* **123**:1081–1088.

Parsons DW, Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu I, Gallia GL, Olivi A, Mclendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Jr LAD, Hartigan J, Smith DR, Strausberg RL, Kazue S, Marie N, Mieko S, Shinjo O, Yan H, Riggins GJ, Bigner DD, Velculescu VE, and Kinzler KW (2008) An Integrated Genomic Analysis of Human Glioblastoma Multiforme. *Science (80-)* **321**:1807–1812.

Pearson FG (1999) Non-small Cell Lung Cancer * Role of Surgery for Stages I-III. *Chest* **116**:500S–503S, The American College of Chest Physicians.

Qian T, Liu Y, Dong Y, Zhang L, Dong Y, Sun Y, and Sun D (2018) CXCR7 regulates breast tumor metastasis and angiogenesis in vivo and in vitro. *Mol Med Rep* **17**:3633–3639.

Rajagopal S, Kim J, Ahn S, Craig S, Lam CM, Gerard NP, Gerard C, and Lefkowitz RJ (2010) -arrestin- but not G protein-mediated signaling by the "decoy" receptor CXCR7. *Proc Natl Acad Sci* **107**:628–632.

Rao S, Sengupta R, Choe EJ, Woerner BM, Jackson E, Sun T, Leonard J, Piwnica-Worms D, and Rubin JB (2012) CXCL12 mediates trophic interactions between endothelial and tumor cells in glioblastoma. *PLoS One* **7**.

Salazar N, Muñoz D, Kallifatidis G, Singh RK, Jordà M, and Lokeshwar BL (2014) The chemokine receptor CXCR7 interacts with EGFR to promote breast cancer cell proliferation. **2**:1–13.

Sánchez-Alcañiz JA, Haege S, Mueller W, Pla R, Mackay F, Schulz S, López-Bendito G, Stumm R, and Marín O (2011) Cxcr7 Controls Neuronal Migration by Regulating Chemokine Responsiveness. *Neuron* **69**:77–90.

Sánchez-Martín L, Sánchez-Mateos P, and Cabañas C (2013) CXCR7 impact on CXCL12 biology and disease. *Trends Mol Med* **19**:12–22.

Shimizu N, Soda Y, Kanbe K, Liu HY, Mukai R, Kitamura T, and Hoshino H (2000) A putative G protein-coupled receptor, RDC1, is a novel coreceptor for human and simian immunodeficiency viruses. *J Virol* **74**:619–26.

Siegel RLRL, Miller DK, Jemal A, Miller KD, and Jemal A (2016) Cancer Statistics, 2016. *CA Cancer J Clin* **66**:7–30.

Sierro F, Biben C, Martı L, Woehl B, Leung H, Groom J, Batten M, Harvey RP, Martı C, Mackay CR, Mackay F, Martínez-Muñoz L, Mellado M, Ransohoff RM, Li M, Woehl B, Leung H, Groom J, Batten M, Harvey RP, Martínez-A C, Mackay CR, and Mackay F (2007) Disrupted cardiac development but normal hematopoiesis in mice deficient in the second CXCL12/SDF-1 receptor, CXCR7. *Proc Natl Acad Sci U S A* **104**:14759–64.

Singh A, and Settleman J (2010) EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. *Oncogene* **29**:4741–4751, Nature Publishing Group.

Stacer AC, Fenner J, Cavnar SP, Xiao A, Zhao S, Chang SL, Salomonnson A, Luker KE, and Luker GD (2015) Endothelial CXCR7 regulates breast cancer metastasis. **35**:1716–1724, Nature Publishing Group.

Stavrovskaya AA, Shushanov SS, and Rybalkina EY (2016) Problems of Glioblastoma Multiforme Drug Resistance. *Biochem Biokhimiia* **81**:91–100.

Tang X, Li X, Li Z, Liu Y, Yao L, Song S, Yang H, and Li C (2016) Downregulation of CXCR7 inhibits proliferative capacity and stem cell-like properties in breast cancer stem cells. *Tumor Biol* **37**:13425–13433, Tumor Biology.

Touat M, Idbaih A, Sanson M, and Ligon KL (2017) Glioblastoma targeted therapy: updated approaches from recent biological insights.

Uhlen M, Zhang C, Lee S, Sjostedt E, Fagerberg L, Bidkhori G, Benfeitas R, Arif M, Liu Z, Edfors F, Sanli K, Feilitzen K Von, Oksvold P, Lundberg E, Hober S, Nilsson P, Mattsson J, Schwenk JM, Brunnström H, Glimelius B, Sjöblom T, Edqvist P, Djureinovic D, Micke P, Lindskog C, Mardinoglu A, and Ponten F (2017) A pathology atlas of the human cancer transcriptome. *Science (80-)* **357**:1–11.

Walters MJ, Ebsworth K, Berahovich RD, Penfold MET, Liu S, Omran R Al, Kioi M, and Chernikova SB (2014) Inhibition of CXCR7 extends survival following irradiation of brain tumours in mice and rats. *Br J Cancer* **110**:1179–1188, Nature Publishing Group.

Wani NA, Nasser MW, Ahirwar DK, Zhao H, Miao Z, Shilo K, and Ganju RK (2014) C-X-C motif chemokine 12/C-X-C chemokine receptor type 7 signaling regulates breast cancer growth and metastasis by modulating the tumor microenvironment. *Breast Cancer Res* **16**:R54.

Wu YC, Tang SJ, Sun GH, and Sun KH (2016) CXCR7 mediates TGFβ1-promoted EMT and tumor-initiating features in lung cancer. *Oncogene* **35**:2123–2132.

Xu C, Fillmore CM, Koyama S, Wu H, Zhao Y, Chen Z, Herter-Sprie G, Akbaym E, Jeremy T, Altabef A, Reibel J, Walton Z, Ji H, Watanabe H, Janne P, Castrillon DH, Rustgi AK, Bass AJ, Freeman

GJ, Padera RF, Dranoff G, Hammerman PS, Kim CF, and Wong K (2014) Loss of Lkb1 and Pten Leads to Lung Squamous Cell Carcinoma with Elevated PD-L1 Expression. *Cancer Cell* **25**:590–604.

Xue TC, Chen RX, Ren ZG, Zou JH, Tang ZY, and Ye SL (2013) Transmembrane receptor CXCR7 increases the risk of extrahepatic metastasis of relatively well-differentiated hepatocellular carcinoma through upregulation of osteopontin. *Oncol Rep* **30**:105–110.

Yu H, Xue Y, Wang P, Liu X, Ma J, Zheng J, Li Z, Li Z, Cai H, and Liu Y (2017) Knockdown of long non-coding RNA XIST increases blood–tumor barrier permeability and inhibits glioma angiogenesis by targeting miR-137. *Oncogenesis* **6**:e303.

Yu PF, Huang Y, Xu CL, Lin LY, Han YY, Sun WH, Hu GH, Rabson AB, Wang Y, and Shi YF (2017) Downregulation of CXCL12 in mesenchymal stromal cells by TGFβ promotes breast cancer metastasis. *Oncogene* **36**:840–849.

Zabel BA, Miao Z, Lai NL, Wang Y, Lewen S, Berahovich RD, Jaen JC, and Schall TJ (2010) CXCR7 protein expression correlates with elevated mmp-3 secretion in breast cancer cells. *Oncol Lett* 845–847.

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Figure 1: Graphical representation illustrating the role of ACKR3 in cancer. See text for details