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REVIEW ARTICLE

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The involvement of a chemokine receptor antagonist CTCE-9908 and kynurenine metabolites in cancer development

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Abstract

Cancer is the second leading cause of mortality worldwide. Skin cancer is the most common cancer in South Africa with nearly 20,000 reported cases every year and 700 deaths. If diagnosed early, the 5-year survival rate is about 90%, however, when diagnosed late, the 5-year survival rate decreases to about 20%. Melanoma is a type of skin cancer with an estimated 5-year survival rate of approximately 90%. Neuroblastoma is a paediatric cancer with a low survival rate. Sixty percent of patients with metastatic disease do not survive 5 years after diagnosis. Despite recent advances in targeted therapies, there is a crucial need to identify reliable prognostic biomarkers which will be able to contribute to the development of more precision-based chemotherapeutic strategies to prevent tumour migration and metastasis. The compound, CTCE-9908 inhibits the binding of CXC chemokine ligand 12 (CXCL12) to the CXC chemokine receptor 4 (CXCR4) receptor leading to reduced metastasis. Kynurenine metabolites are derived tryptophan, which is an essential amino acid. Kynurenine metabolites inhibit T-cell proliferation resulting in cell growth arrest. For this reason, chemokines receptors represent potential targets for the treatment of cancer growth and metastasis. In this review paper, the role of the CXCL12/CXCR4 signalling pathway in the development of cancer is highlighted together with the current available treatments involving the CTCE-9908 compound in combination with microtubule inhibitors like paclitaxel and docetaxel.

KEYWORDS

CTCE-9908, kynurenic acid, L-Kynurenine, melanoma, neuroblastoma, quinolinic acid

Danielle Sandra Nkandeu and Charlize Basson contributed equally to this work.

1 | INTRODUCTION

Cancer is the second leading cause of mortality worldwide. An estimated 19.3 million new cancer cases have been reported in 2020 with approximately 10 million deaths worldwide.[1] Skin cancer is the most common malignancy in the world affecting both men and women.[2] According to the Cancer Association of South Africa (CANSA), South Africa has the second-highest incidence of skin cancer in the world after Australia.[3] South Africa recorded 107,467 cancer cases in 2018 with 57,373 cancer-related deaths.[4] Skin cancer is the most common cancer in South Africa with nearly 20,000 reported cases every year and 700 deaths.[4]

Melanoma is a type of skin cancer that occurs when pigment-producing cells found on the upper layer of the skin called melanocytes mutate and begin to divide rapidly and uncontrollably.[4] The World Health Organization (WHO) reports that approximately two to three million nonmelanoma skin cancers namely squamous cell carcinoma and basal cell carcinoma and nearly 132,000 malignant melanomas occur worldwide every year.[4] The incidence for melanoma in South Africa is 2.7 per 100,000 individuals with 51% of cases reported in males and 48% reported in individuals older than 60 years.[4]

Melanoma can manifest in various organs and can be categorised as mucosal melanoma, ocular or uveal melanoma or cutaneous melanoma, where the latter is the most prevalent type (in more than 90% cases).[5,6] Cutaneous melanoma represents an increasing world health problem. Franco et al. demonstrated an increase in CXCR4 expression in uveal melanoma tumours which correlated with disease progression.[7,8] Despite intensive research, there is currently no cure for metastatic disease, which commonly targets the liver and results in a 5-year survival rates of less than 12%.[9]

Despite recent advances in targeted therapies, there are still no reliable treatments or biomarkers to assist with disease progression. Therefore, there is a crucial need to identify reliable prognostic biomarkers which will be able to contribute to the development of more precision-based chemotherapeutic strategies to prevent tumour migration and metastasis.[2]

Neuroblastoma (NB) is the most common extracranial solid tumour observed in childhood with an average diagnosis age of 17 months.[10,11] NB causes 12% of childhood cancer mortality despite representing just 5% of all paediatric cancer diagnoses.[5,6] The disease is very advanced at the time of diagnosis; as children are mostly diagnosed at Stage 4 with a 5-year survival rate of 26%.[10,11] Treatment at Stage 4 includes chemotherapy and primary tumour resection.[10,11] Half of the patients relapse after receiving treatment.[10,11] Overall survival is about 80% but decreases to 50% for children with high-risk disease.[5,6]

Recent developments have been made in chemotherapy, immunotherapy and radiation therapies to improve current treatment strategies. While these developments and improvements show promising results there is still possibility of relapse.[12] Therefore a novel chemotherapeutic treatment aimed at improving survival rates of children diagnosed with NB is warranted.

Significance statement

- The compound, CTCE-9908 inhibits the binding of the CXCL12 ligand to the CXCR4 receptor leading to reduced metastasis.
- Kynurenine metabolites inhibit T-cell proliferation resulting in cell growth arrest. Chemokines receptors, therefore, represent potential targets for the treatment of cancer growth and metastasis.
- In this review paper, the role of the CXCL12/CXCR4 signalling pathway in the development of cancer is highlighted together with the current available treatments involving the CTCE-9908 compound.

Chemokines and their receptors play a critical role in cellular homeostasis namely, embryogenesis and immune response.[13] Chemokines promote the migration of chemokine receptor-expressing cells down chemokine ligand gradients.[12] In cancer cells, the upregulation of chemokine receptors results in metastasis via the CXCL12/CXCR4 signalling pathway.[13] An elevated alpha-chemokine receptor C-X-C chemokine receptor 4 (CXCR4) expression has been observed in melanoma resulting in disease progression and metastasis.[14] The increase in CXCR4 expression in melanoma cells promotes migration towards CXCL12 which is expressed in human tissues including the liver, lungs, bone marrow and lymph nodes.[14] The CXCL12/CXCR4 signalling promotes tumour cell proliferation and survival through the paracrine-mediated activation of MAPK cell signalling.[14] In NB, CXCR4 is expressed in primary tumours.[15] Evidence has linked the CXCL12/CXCR4 axis to tumour migration toward the bone marrow resulting in higher frequency of metastasis in vivo.[14] Furthermore, it has been shown that overexpression of CXCR4 in NB may increase primary and metastatic growth.[15] This review focuses on the role of chemokines in melanoma and NB and possible therapeutic strategies such as CTCE-9908 and kynurenine metabolites to alter chemokine activation.

2 | MELANOMA

Melanoma is an aggressive cancer when detected late.[4] If diagnosed early, the 5-year survival rate is about 90%, however, when diagnosed late, the 5-year survival rate decreases to about 20%.[4] The incidence of melanoma in South Africa is 2.7 per 100,000 individuals with 51% of cases reported in males and 48% reported in individuals older than 60 years.[4]

2.1 | Stages of melanoma cancer

The stages of melanoma provide an indication of tumour thickness, presence of ulceration and the presence of mitosis in lesions less than

1 mm in thickness.[16] In Stage 0 or melanoma in situ, the cancer is confined to the epidermis, the outermost skin layer. In the latter stage, it has not spread to lymph nodes. In Stage I, the cancer is about 2 mm thick and might not ulcerated. Stage II melanoma is characterized by tumour size of 4 mm or above; the tumour has not spread to lymph nodes or other organs. In Stage III, the cancer is above 2 mm thick and might be ulcerated. The cancer has spread to 1 to 3 nearby lymph nodes, but it is so small that it can only be visualised via microscopy. Stage IV melanoma occurs when the cancer has spread to distant lymph nodes and to organs like the lungs, the liver or the brain.[16]

2.2 | Types of melanoma cancer

There are four main types of skin melanoma, the superficial spreading melanoma, lentigo maligna, acral lentiginous melanoma and nodular melanoma.[3,4,13] The superficial spreading melanoma is the most common form of melanoma. It may originate in an existing mole or may appear as a new skin lesion.[3,4,13] When it begins in an existing mole, it tends to grow on the surface of the skin for some time before penetrating the deeper layers of the skin.[3,4,13] It can be found anywhere on the body, however, it is most likely to appear on the torso in men and on legs in women.[3,4,13] It may appear as a flat or slightly raised and discoloured asymmetrical patch with uneven borders. The colours range from shades of tan, black, red, pink, blue or white. It may also lack pigment and appear as a pink or skin-tone lesion.[3,4]

Lentigo maligna often develops in older people.[13] Lentigo maligna melanoma develops after lentigo maligna becomes invasive or spreads beyond the original site.[13] This form of melanoma is similar to the superficial spreading melanoma as they both grow close to the skin surface at first.[13] The tumour develops on sun-damaged skin of the face, ears, arms or upper torso.[13] The skin lesion may look like a flat or slightly raised blotchy patch with uneven borders. The colour is usually blue, black, tan or brown.[13] The acral lentiginous melanoma is the most common form of melanoma found in people of colour including individuals of African ancestry.[3,4] It often appears in hidden places like under the nails and on the soles of the feet or in the palms of the hands. It may appear as a black or brown area of the skin.[3,4] The nodular melanoma is the most aggressive type of melanoma. It accounts for 15%–20% of all cases in South Africa.[3,4] Nodular melanoma can be identified by the formation of mole in the skin usually blue or black in colour, but it may also appear pink or red.[3,4] The tumour grows deeper into the skin more rapidly than other melanoma types and it is most frequently found on the torso, legs and arms as well as in the scalp of older men.[3,4] It is usually invasive at the time it is first diagnosed it has already reached the deeper layers of the skin.[3,4] The melanoma (B-16 F10) cell line is a skin melanoma cell line derived from embryonic stem cells.[2] The B-16 F10 was derived from the B-16 parent tumour line in C57BL/6 mice.[2] It has been reported that the B-16 F10 cells express CXCR4, which has been linked to poor prognosis and metastasis.[2]

3 | NB

NB is a paediatric cancer with a low survival rate with 60% of patients with metastatic disease who do not survive 5 years after the diagnosis.[10,11,14] NB affects approximately 8 per 1,000,000 children with nearly 600–700 cases diagnosed in the United States. In northern Africa, NB accounted for 47% of childhood cancer while in the sub-Sahara, it represented 5% of childhood cancer.[12] NB causes 12% of childhood cancer mortality despite representing just 5% of all paediatric cancer diagnoses.[10,11] Overall survival is about 80% but decreases to 50% for children with high-risk disease.[10,11]

NB presents as a clinical and heterogenous tumour of embryonic origin that comes from primitive neural crest cells.[10,11,14] It is the most common cancer diagnosed during the first year of life.[10,11,14] This childhood malignancy usually presents as abdominal, thoracic or neck masses originating in the adrenal medulla or paraspinal sympathetic ganglia and is often metastatic at the time of diagnosis.[10,11] NB tumours are stratified into low-, intermediate- and high-risk groups based on clinical, histologic and genomic characteristics.[10,11]

There are four main stages of NB cancer, Stage 1, Stage 2, Stage 3 and Stage 4. During Stage 1, the tumour is in one area of the body and has not spread yet. The cancer can be removed completely with surgery.[11] Stage 2 is divided into 2A and 2B. In Stage 2A, the tumour is located in one area, however, it cannot be completely removed with surgery because of its size or position.[11] The surrounding lymph nodes do not contain any cancer cells. In Stage 2B, the cancer is still located in one area; it may or may not be possible to completely remove the cancer with surgery. The tumour has spread to nearby lymph nodes.[11] Stage 3 means that the tumour cannot be removed by surgery and it has spread to lymph nodes near the tumour or to other areas near the tumour, but it has not spread to other parts of the body.[11] When the original tumour spreads to distant lymph nodes, bones, bone marrow and liver the tumour is in Stage 4.

Stage 4S is a special case, it diagnosed in infants younger than 1 year. In this instance, the tumour has spread to the skin, liver and bone marrow.[11] Usually, less than 10% of cells examined are cancerous.[11] Children with Stage 4S almost always get better with very little treatment. The tumours either regress spontaneously or after administration of chemotherapeutic drugs only if there are symptoms.[11] The International Neuroblastoma Risk Group Staging System (INRGSS) uses the results of imaging tests to determine the stage of the disease. There are also four stages namely, Stage L1, Stage L2, Stage M and Stage MS.[11] In Stage L1, the tumour is localised and it has not spread to other vital organs near the tumour. No image-defined risk factors (IDRF) are identified. The tumour can be removed by surgery.[11] During Stage L2, the tumour is localised and it has not spread beyond the area where it started. However, there are IDRFs found on the image and the tumour cannot be removed safely by surgery. Once the tumour has spread to other parts of the body, it is in stage M. Stage MS also called the metastatic cancer stage, occurs when the tumour has spread to the skin, liver and bone marrow in children younger than 18 months old.[11]

Human NB cells (SH-SY5Y) have the ability to differentiate into neurons; they have therefore been widely used as the in vitro cell

culture model for neuroscience model. Cellular elasticity could serve as a potential marker to quantitatively distinguish between undifferentiated and differentiated SH-SY5Y cells.[14] Neuronal differentiation entails the formation and extension of neuritic processes, increased electrical excitability of the plasma membrane, and formation of synaptophysin-positive functional synapses and induction of neuro-specific enzymes, neurotransmitters and neurotransmitter receptors.[14]

The undifferentiated NBs are characterised by neuroblast-like, nonpolarised cell bodies with few processes. They tend to grow in clusters and may form clumps as the cells appear to grow on top of each other. The human NB SH-SY5Y cell line has been used extensively to investigate the neuronal response after exposure to toxins in Parkinson's disease (PD). A NB cell line (SH-SY5Y) is differentiated through the addition of retinoic acid (RA) to the cell culture medium. Upon stimulation by RA, the SH-SY5Y cells can differentiate into nonproliferating neuronal cells.[14] Evidence suggests that RA-differentiated NB cells are more appropriate to study neurotoxicity in PD research. Undifferentiated SH-SY5Y's characteristics include, neuroblast-like morphology with non-polarised cell bodies and few processes.[14] The highly metastatic characteristic of undifferentiated SH-SY5Y cells make them more suitable for in vitro cancer research as they are also able to continuously proliferate.[14] The tumour metastasis and the disease stage of NB are associated with chemokine overexpression, such as CXCR4.[17]

4 | THE ALPHA CHEMOKINE FAMILY

4.1 | The physiological role of alpha-chemokine receptor CXCR4

Chemokines are a family of small molecules (8–10 kDa). Chemoattractive cytokines play active roles in embryogenesis, hematopoiesis, mitogenicity as well as innate and adaptive immunity.[18] Chemokines are divided into CXC, CX3C, CC and C subfamilies depending on different cysteine residues at the N-terminal.[18] Chemokines bind and activate receptors such as the G-protein coupled receptors (GPCR) that are found on the target cells. Over 50 chemokines and their corresponding receptors have been identified.[13,18]

Functionally, chemokines can be divided into two groups, which include inflammatory and homeostatic chemokines.[19] Chemokine receptors were originally discovered on leukocytes and found to endow leukocytes with the ability to migrate towards inflammatory sites,[20] which gave rise to their well-known inflammatory properties.[19] In contrast, homeostatic chemokines are implicated in haematopoiesis.[19] Haematopoiesis is regulated by directional migration of haemopoietic stem cells (HSC), located in microenvironmental units called niches,[21] to bone marrow stromal cells.[22] Haemopoietic stem cells (HSC) express CXCR4[23] and are highly responsive to CXCL12.[24] Expression of CXCR4 on HSC and CXCL12 on the stromal cells of the bone marrow leads to the formation of the migration gradient.[23] The homing of CXCR4 to CXCL12 may be attributed to bone marrow specific homing of HSC, with the purpose to retain HSC in a bone marrow niche for proliferation, differentiation,[22] self-renewal and survival.[25] To home to the specific niche, HSC express cell adhesion receptors to facilitate adhesion in a cell–cell or cell–extracellular matrix (ECM) manner.[21] These adhesion receptors' affinity to their ligands can be regulated to adapt to the HSC demand.[21]

Recent research suggests that chemokines serve multiple roles through interaction with their receptors.[26] These roles not only refer to physiological functions such as homeostatic and inflammatory responses,[27] but also pathological roles such as the activation of several signalling pathways in cancer to promote tumour cell migration, proliferation, survival and adhesion.[28–30]

4.2 | The role of alpha-chemokine receptor CXCR4 in cancer

It has been shown that chemokines and chemokine receptors play a crucial role in cancer metastasis including melanoma and breast cancer.[2] The alpha-chemokine receptor CXCR4 was identified as the most widely expressed in cancers including oral cancer, oesophageal cancer, gastric cancer, colon cancer, liver cancer, breast cancer and melanoma and involved in cell migration and proliferation as well as in tumour metastasis.[18] The corresponding ligand, CXCL12 was first defined as a lymphocyte chemoattractant and was known to regulate haematopoiesis [28] by trafficking lymphocytes to the bone marrow.[28] More recently, the leukocyte trafficking mechanism was found to be similar to tumour cell migration and metastasis.[31] In vitro experiments have substantiated that the interaction between CXCR4 and CXCL12 promote directional migration of cancer cells, including melanoma and NB.[17,32]

This ligand is produced by stromal as well as cancer cells.[33] Tumour-associated CXCL12 expression attracts CXCR4 expressing inflammatory, vascular and stromal cells to the tumour mass [34] and stimulates proliferation and invasion in an autocrine manner (Figure 1).[30] The recruited CXCR4-expressing cells promote primary tumour proliferation through the secretion of growth factors, cytokines, chemokines and proangiogenic factors.[30,34] CXCL12-secreting stromal fibroblasts are attracted by CXCR4 expressing tumour cells to retain tumour cells within the microenvironment.[35] The tumour cells are retained within this cellular microenvironment, which promotes cancer cell survival and proliferation.[22]

This CXCL12/CXCR4 interaction between stromal and cancer cells within the microenvironment, leading to tumour-associated CXCR4 activation, is also referred to as paracrine signalling (Figure 1).[30] Paracrine signalling is responsible for the regulation of primary tumour growth and metastasis.[30] Metastasis is evoked by CXCR4 expressing tumour cells, which migrate to organs expressing a greater amount of its corresponding ligand CXCL12.[37,38] The binding and activation of CXCR4 by the corresponding ligand CXCL12, predominantly expressed by stromal cells such as lymphatic endothelial cells,[39] previously promoted metastasis by directing

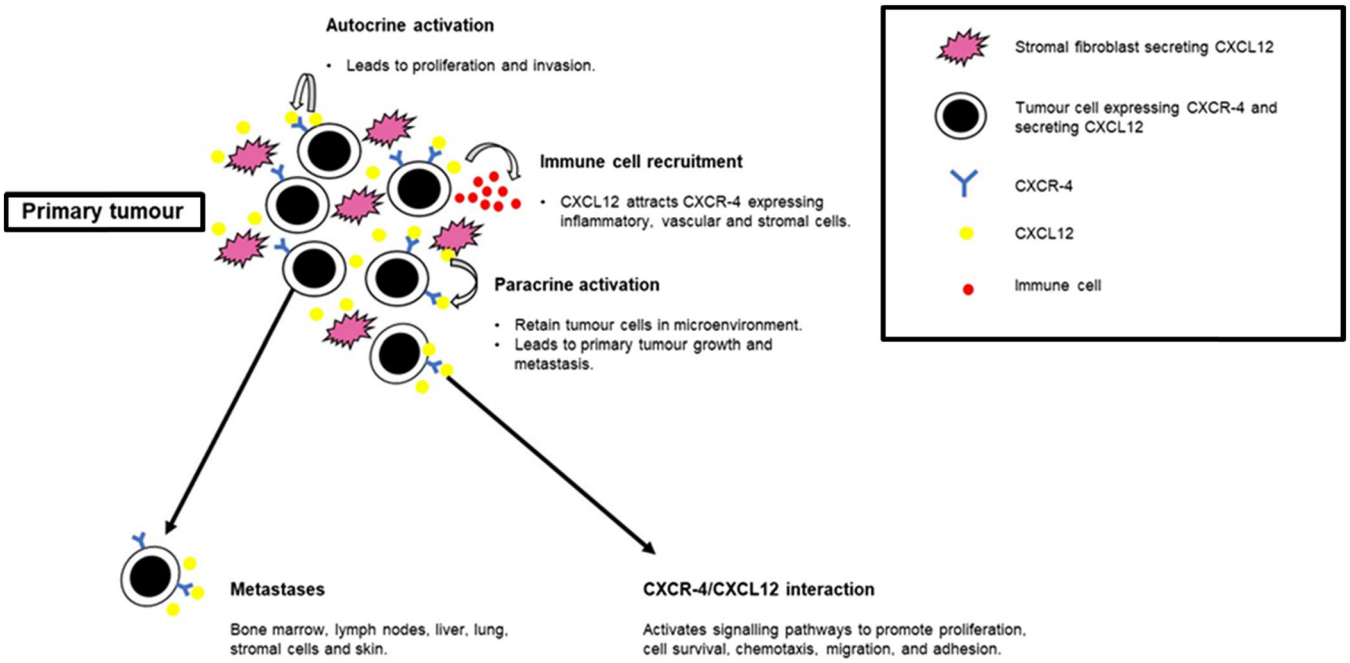

FIGURE 1 Schematic diagram of the possible roles of CXCR4 and CXCL12 signalling in cancer.[14,30,33–36] CXCR4 activation by CXCL12 can occur via autocrine or paracrine activation. CXCR4 activation may then lead to metastasis to other organs and the activation of signalling pathways to promote proliferation, cell survival, chemotaxis, migration and adhesion. (This image was designed using PowerPoint Microsoft office 365). CXCR4, CXC chemokine receptor 4; CXCL12, CXC chemokine ligand 12.

melanoma tumour cells towards target organs.[40] The association of CXCR4 with metastasis and tumour cell growth, may lead to promising therapeutic strategies involving CXCR4.[41] Furthermore, the CXCL12/CXCR4 interaction activates several signalling pathways in target cells [38] to regulate cell processes such as proliferation, cell survival, migration, and adhesion (Figure 1).[33,42] The CXCL12/CXCR4 axis plays a crucial role in promoting and maintaining cancer cells and has therefore emerged as a promising target for anticancer chemotherapy.[18]

5 | SIGNALLING PATHWAYS OF THE CXCL12/CXCR4 AXIS

The chemokine receptor CXCR4 is a G-protein-coupled receptor (GPCR).[43] Upon activation of CXCR4 by CXCL12, the receptor undergoes a conformational change, which activates arrestins and the G-protein heterotrimer.[44] G-protein-coupled receptors (GPCR) heterotrimers consist of Gα, Gβ and Gγ subunits, which upon activation, favours the replacement of guanosine diphosphate (GDP) with guanosine triphosphate (GTP).[45] The GDP/GTP exchange facilitates the dissociation of the Gα subunit from the Gβγ dimer,[46] enabling both components to participate in various signalling cascades.[28] Highlighted signalling pathways of the CXCL12/CXCR4 axis include activation of the phospholipase C (PLC) pathway; the mitogen-activated protein kinase (MAP-K) pathway; the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway[47] and adhesion proteins [integrins,[48] focal adhesion kinase (FAK)[49] and paxillin (PXN)].[50]

The signalling of the CXCL12/CXCR4 axis is regulated by receptor internalization and lysosomal degradation.[28] This phenomenon was proven multiple times by the nuclear, cytoplasmic and membrane CXCR4 staining in tumour cells.[34] Upon activation, CXCR4 signal is then rapidly desensitized[29] and the cytoplasmic segment of the chemokine receptor (CXCR4) is phosphorylated by G-protein-coupled receptor kinase (GRK).[29,46] This enables β-arrestin to effectively bind to CXCR4 and uncouple it from its ligand (the G-protein).[47] β-arrestin mediates internalization CXCR4,[51] thereby making it susceptible for lysosomal degradation (Figure 2).[46]

5.1 | Protein kinase C (PKC) signalling pathway

G-protein-associated activation of $G\alpha_q$, leads to the activation of PLC (specifically PLCβ) to initiate the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) in the phospholipid bilayer.[28,51,52] Literature suggests that the Gβγ dimer might also be responsible for PLC activation,[29] but a consensus has not been reached.[51] The secondary messenger IP_3 binds to ligand-mediated calcium (Ca^{2+}) receptors on the endoplasmic reticulum (ER) to induce Ca^{2+}-influx into the cytoplasm, whereas DAG activates protein kinase C (PKC).[28] The generation of these two end products consequently gives rise to elevated cytoplasmic Ca^{2+} and cell migration (Figure 2).[47]

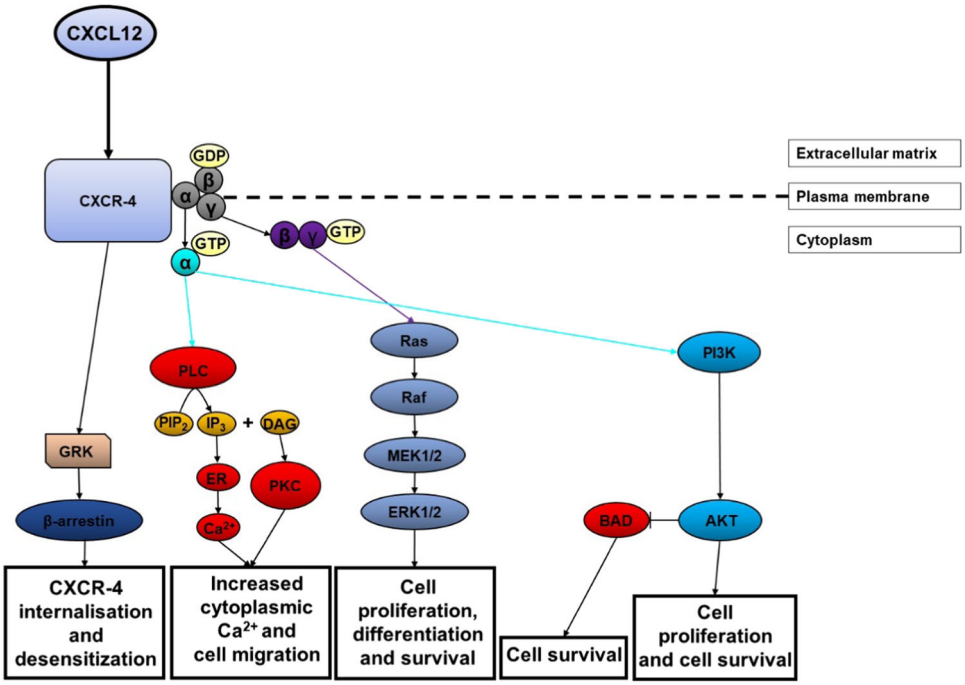

FIGURE 2 Illustration of proposed signalling pathways of the CXCL12/CXCR4 axis. (This image was designed using PowerPoint Microsoft office 365). AKT, protein kinase B; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; Ca^{2+}, calcium ions; CXCL12, CXC chemokine ligand 12; CXCR4, CXC chemokine receptor 4; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; GDP, guanosine diphosphate; GRK, G-protein-coupled receptor kinase; GTP, guanosine triphosphate; MAP-K, mitogen-activated protein kinase; MEK1/2, MAP-K/ERK kinase1/2; PIP_2, phosphatidylinositol bisphosphate; IP_3, inositol-1,4,5-trisphosphate; DAG, diacylglycerol; PKC, protein kinase C; PLC, phospholipase C; PI3K, phosphoinositide 3-kinase; Raf, rapidly accelerated fibrosarcoma; Ras, rat sarcoma virus.

5.2 | MAP-K signalling pathway

The MAP-K pathway has received overwhelming attention in cancer research, as this pathway is dysregulated in up to 80% of melanomas[53] and 61% of NBs.[54] The activity of the MAP-K pathway is regulated by all four of the G-protein families including G_s, G_i, G_q and G_{12}.[55] The MAP-K pathway is comprised of four subpathways,[56] which can be activated by both the Gβγ dimer and the α subunit.[55] Upon the activation of CXCR4, the Gβγ complex acts as a functional subunit to stimulate MAP-K modules, such as Ras to activate the ERK1/2 MAPK cascade.[52] The MAP-K signalling cascade follows an upstream enzymatic sequence of mitogen-activated protein kinase-kinase-kinase (MAP-KKK), mitogen-activated protein kinase-kinase (MAP-KK) and MAP-K, where each module acts as an enzyme.[56,57] Activated MAP-K consequently moves to the nucleus to control gene expression through the phosphorylation of transcription factors.[55,56] The activated MAP-K sub-pathways ultimately affects cell proliferation, differentiation and apoptosis (Figure 2).[55,56]

5.3 | PI3K/AKT signalling pathway

Both Gβγ and Gα subunits have the ability to initiate the PI3K pathway,[18,29,38,58] which plays a key role in the phosphorylation of multiple focal adhesion (FA) components including proline-rich kinase-2 (Pyk-2), Crk-associated substrate (p130Cas), FAK, PXN, Nck, Crk and Crk-L.[18,29] Activated FA components contribute to actin cytoskeletal rearrangement and cell migration.[18,38,58] Furthermore, the induction of the PI3K pathway leads to the phosphorylation and subsequent activation of AKT (also called protein kinase B),[18,28] to induce cell proliferation and survival.[29] The AKT signalling cascade may inactivate of the proapoptotic BCL-2-associated death,[18,59] which may lead to inhibition in cytochrome *c* from the mitochondria and caspase 3 activation.[60] The AKT pathway can thus ultimately result in cell survival (Figure 2).[18,59]

6 | ADHESION PROTEINS

Numerous cell types require integrin-mediated attachment to the ECM for growth and survival. The binding of integrins to the ECM results in the phosphorylation of PXN and FAK. In addition, integrins stimulate PXN tyrosine phosphorylation by FAK at FAs.[61,62]

6.1 | FAK

FAK and PXN are FA-associated proteins that play a role in transmitting signals downstream of integrins. Those signals control biological events like cell migration, proliferation and survival.[61] The

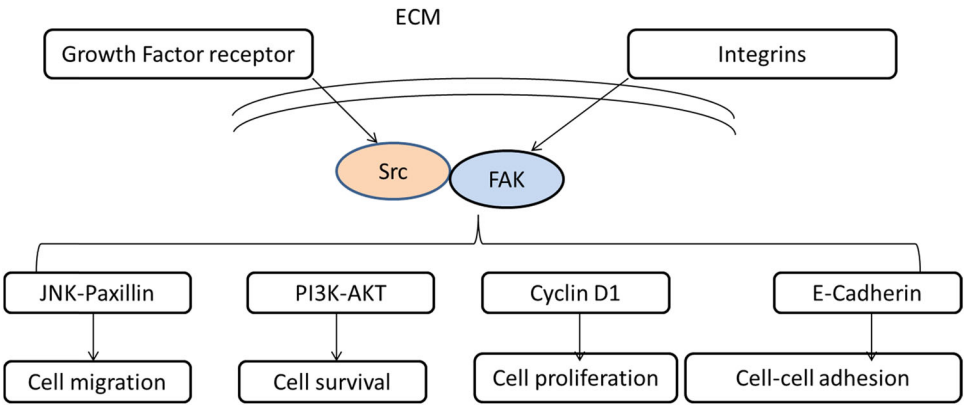

FIGURE 3 Focal adhesion kinase (FAK) signalling pathway in the development of cancer. The activation of FAK is initiated by integrins associated to the extracellular membrane (ECM) and by binding to growth factors receptors. The FAK-Src complex binds and initiates cell migration, cell survival, cell proliferation as well as invasion and metastasis which ultimately result in cancer cell development.[62] (This image was designed using PowerPoint Microsoft 2016 and saved as a tiff image). PI3K/AKT, phosphoinositide 3-kinase/protein kinase B.

FAK-Src complex binds to and phosphorylates adaptor proteins like PXN. In healthy cells, the activated FAK-Src complex stimulates cell survival, cell motility and cell cycle progression (Figure 3).[61] Studies have shown that FAK promotes the development and progression of cancer.[61] Wang et al.[62] reported that FAK was associated with cancer progression and metastasis.[62] During anoikis, a programmed cell death due to disruption in cell–ECM interactions, FAK activity is inhibited resulting in apoptosis.[61,62] Overexpression of FAK in cancer may contribute to the resistance to anoikis. In cancer, there is an increase in the FAK/Src complex which leads to the activation of the PI3K-AKT as well as the MEK-extracellular signal-regulated kinase 1/2 (ERK1/2) signalling pathways, therefore, increasing cancer cell growth and survival (Figure 3).[61,62] Transforming growth factor-β (TGF-β1) also plays a role in the activation of AKT and FAK via the SMAD3 and the p38 MAPK pathways resulting in tumour progression and anoikis resistance. Moreover, overexpression of FAK has been shown to inhibit caspase 3-mediated apoptosis conversely, the inhibition of FAK leads to apoptosis in cancer.[62] FAK also binds to the death domain kinase receptor-interacting protein (RIP), a component of the death receptor complex, therefore, inhibiting apoptosis by suppressing the function of the death domain of RIP.[62]

FAK is a key regulator in promoting cancer cell proliferation. Overexpression of FAK increases cyclin D1 expression and decreases p21 expression which is a cyclin-dependent kinase inhibitor, therefore, accelerating G1 to S phase transition.[61,62] Evidence suggests that any change in the adhesion properties of neoplastic cells may lead to the development and progression of cancer. Loss of intercellular adhesion will allow the malignant cells to escape from their site of origin, degrade the ECM, acquire a more motile phenotype and finally invade and metastasize.[61,62] Moreover, adhesion molecules play a crucial role in signal transduction, cell growth and differentiation, site-specific gene expression, cell motility, wound healing and inflammation.[61,62]

6.2 | PXN

PXN, a main component of FAs, plays a crucial role in the transduction of the extracellular signals into intracellular responses triggered after the binding of integrins to the ECM.[61] PXN contributes as a scaffolding protein to the recruitment of specific kinases, phosphatases, oncoproteins and structural proteins involved in intracellular signalling pathways.[61] The activation of these pathways leads to the reorganization of the actin cytoskeleton assembly of FAs required for cell attachment, proliferation and migration (Figure 4). PXN also plays a role in the disassembly of FAs during cell movement and migration. PXN has a positive and negative effect on cell migration.[61]

The PXN family genes include PXN, Hic-5 and leupaxin (LPXN), they share binding sequences for several interacting proteins but they have different functions.[61] PXN is expressed in most tissues of the nervous system.[61] Hic-5 is expressed in the smooth muscle of the blood vessels.[61] LPXN expression was initially found in leukocytes, however, it was recently identified in cells from diverse lineages.[61] The lack of expression of PXN during embryonic development is lethal whereas the deletion of Hic-5 results in minor changes in the vascular system development.[61]

PXN acquires gain of function mutations that are associated with tumour progression including breast, lung, prostate, melanoma and colorectal cancer.[61] Recent studies have demonstrated that overexpression of PXN at the RNA and protein level is associated with glioblastoma multiforme tumour malignancy and poor prognosis.[61]

6.3 | Integrins

Cancer progression is associated with complex signalling between cancer cells and the surrounding ECM[63] and all stages of cancer progression, including metastasis, involve cell adhesion.[64,65] Cell

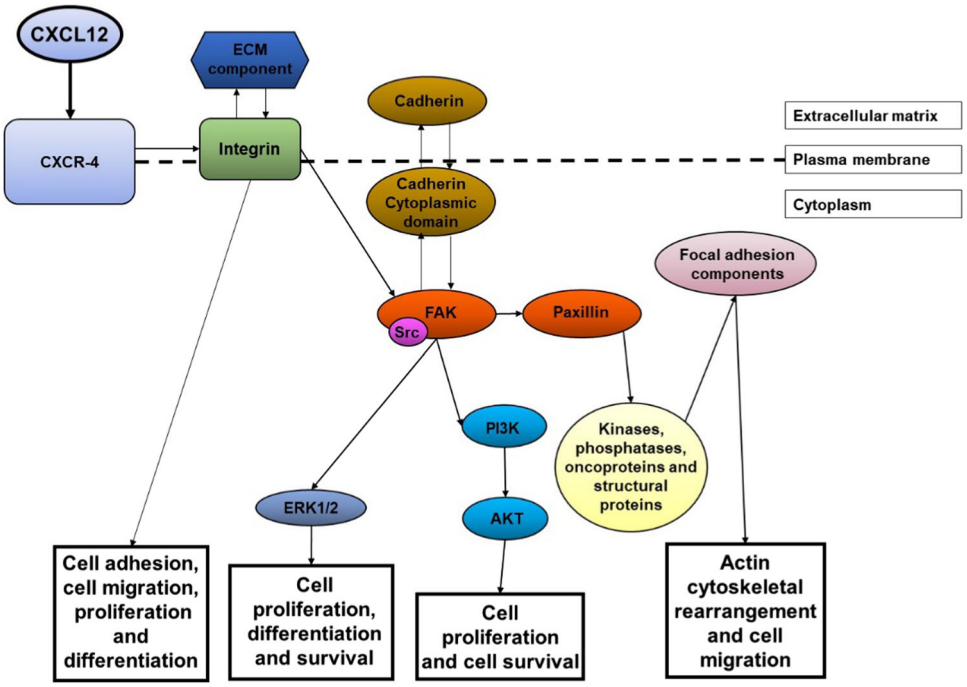

FIGURE 4 The activation of adhesion proteins by CXCR4. Focal adhesion-associated proteins, such as FAK and paxillin play a role in transmitting signals downstream of integrins. The Src-FAK complex not only phosphorylates paxillin but also activates various downstream cancer-associated signalling pathways. Paxillin is a scaffolding protein for kinases, phosphatases, oncoproteins and structural proteins. The activation of these pathways leads to the reorganization of the actin cytoskeleton assembly of focal adhesions required for cell attachment, proliferation, and migration.[18,38,58,61] (This image was designed using PowerPoint Microsoft office 365). AKT, protein kinase B; CXCL12, CXC chemokine ligand 12; CXCR4, CXC chemokine receptor 4; ERK, extracellular-signal-regulated kinase; FAK, focal adhesion kinase; PI3K, phosphoinositide 3-kinase.

adhesion refers to the attachment of one cell to another, thereby providing anchorage for circulating cells.[66,67] Adhesion involves cell adhesion molecules (CAM's),[66] which can be categorised in five types, such as cadherins, selectins, the immunoglobulin superfamily and integrins.[68] Integrins are heterodimeric transmembrane glycoproteins[69] and are functionally categorised as one of five types of CAM's.[68] Integrins contains an α and β subunit.[69] The human integrin network is comprised of 18 α subunits and 8 β subunits to form 24 different integrins with different structural and functional properties.[70,71] Both subunits are required to exert its function, as the α subunit regulates integrin-ligand specificity[72] and the β subunit anchors the cytoskeleton to elicit a plethora of signalling pathways.[72] These signalling pathways mediate several cellular responses such as cell differentiation, migration, survival, cell cycle progression, actin reorganization, migration, gene expression and cell adhesion.[68,73,74] The adhesive and migratory capability of cells can be attributed to integrin expression levels on cell surfaces.[64]

In cancer, the tumour cells rely on the same integrin-mediated processes to promote tumour cell growth and proliferation and to create a microenvironment that is conducive to tumour growth and metastasis.[26] FAK plays a role in β-integrin signalling and together with Src kinase coordinates adhesion, actin cytoskeleton dynamics and cell shape. The FAK/Src kinase complex plays a role in cancer cell migration and cell invasion.[26] Several clinical trials are currently underway involving FAK (GSK2256098, PF04554878 and VS-4718) and Src inhibitors (dasatinib).[26]

7 | INTEGRIN REGULATION

Integrin proteins have interconnected intracellular and extracellular domains.[74] The extracellular domain is involved in integrin-ligand interactions and the intracellular domain bind cytoskeletal proteins.[74] These interactions induce bidirectional integrin signalling, which can be divided into two categories, namely 'inside-out' signalling and 'outside-in' signalling.[75] Integrins do not display constitutive activity[73,76] and have an inactivated or bent conformation with salt bridges linked to the cytoplasmic domains.[77] Integrins must, therefore, be activated in a process called 'inside-out' signalling.[68,73] The 'inside-out' signalling mechanism is initiated by GPCR to modulate integrin affinity[73] and ultimately enables successful integrin–ECM–ligand interactions.[78] Integrins can be activated by the CXCL12/CXCR4 axis, but also directly by CXCL12 that binds to the allosteric site of the integrin.[79] Integrin activation is initiated when integrin adaptor proteins, such as talin or kindlin bind to the β-integrin intracellular tail.[78] This interaction destabilises the salt bridge attached to the cytoplasmic domains,[77] which leads to conformational changes in the protein[73] and the upregulation of the integrin's

binding affinity for the extracellular ligand.[74,78] Activated integrins display an extended conformation, which promotes interaction with the ECM.[80] This cytoplasmic signal-mediated integrin affinity modulation has been proven in β1, β2, β3 and β7 integrins in melanoma.[73]

Upon activation, integrin proteins generate 'outside-in' signalling by binding to a component of the ECM, such as collagen or fibronectin,[73] which is followed by conformational changes of the integrin.[78] These conformational changes are associated with the generation or exposure of binding sites on the integrin.[72] The conformational changes of the integrin are followed by integrin clustering.[78] Clustering of integrins on the membrane leads to the recruitment of various signalling and adaptor proteins to form organized multiprotein complexes on the intracellular side, called FAs.[67,74] These FAs link to the cytoskeleton to mediate responses, such as cell adhesion and migration through the activation of downstream signalling molecules.[74] Integrins can also activate signalling pathways, such as the MAP-K signalling pathway by recruiting signalling components in a direct or indirect manner[80] as well as the PI3K/Akt pathway.[63]

8 | INTEGRIN EXPRESSION AND ACTIVATION

It has previously been indicated that CXCL12 can upregulate integrin expression[79] and modulate its function, which subsequently promotes tumour cell adhesion.[33] Activated integrin proteins are associated with enhanced cell adhesion and invasion in vitro, and experimental metastasis in vivo.[48] The CXCL12/CXCR4 interaction involves integrin proteins, such as very late activation antigen-4 (VLA-4; α4β1), VLA-5 (α5β1).[73] The expression of VLA-4 and VLA-5 is associated with metastasis and poor patient prognosis.[81]

8.1 | VLA-4

Apart from its physiological expression on resting lymphocytes, eosinophils and monocytes,[82] VLA-4 is also expressed on cancer cells, such as melanoma.[83] The adherence of VLA-4 to its ligand, vascular cell adhesion molecule-1 (VCAM-1),[73] is essential for the extravasation of tumour cells during the metastatic process.[84] It was previously demonstrated that CXCR4 activation led to an enhanced affinity of β1 integrins for VCAM-1 in B16 cells.[85] It has been shown that VCAM-1 can also bind to VLA-4 expressed on lymphatic endothelial cells to promote metastasis.[72] The binding of VCAM-1 to VLA-4 stimulates intracellular endothelium Ca^{2+} release, while affecting Ca^{2+} channels and Rac1, all of which is essential to activate endothelial cell nicotinamide adenine dinucleotide phosphate oxidase (NOX2).[86] Upon activation, NOX2 generates superoxide, which dismutates to hydrogen peroxide.[87] Hydrogen peroxide ultimately activates MMP's, to facilitate ECM degradation and subsequent transendothelial cell migration.[88]

8.2 | VLA-5

VLA-5 is expressed in various cancers, including melanoma[89] and plays a crucial role in metastasis.[90] The upregulation of VLA-5 was previously demonstrated in primary and metastatic melanoma cells and was associated with liver metastasis.[91] Melanoma metastasis and protection against apoptosis was previously promoted by enhanced cell adhesion of VLA-5 to fibronectin.[90] A previous study demonstrated a decrease in cell adhesion and PI3K activation upon VLA-5 inhibition in a colon cancer model.[92] In another study in a hepatic cancer system, the inhibition of VLA-5 demonstrated a role in ERK1/2 and p38MAP-K signalling cascades by inducing cell invasion.[92]

9 | THE KYNURENINE PATHWAY

The kynurenine pathway was first discovered in 1853 in the excreted products of animals that were fed tryptophan.[18,93,94] Tryptophan is an essential amino acid used to build proteins and is the starting molecule for the biosynthesis of melatonin and serotonin.[18,93,94] The alternative pathway of tryptophan metabolism occurs via the kynurenine pathway thereby producing biologically active metabolites, the kynurenines. Little is known on the tryptophan metabolism through the kynurenine pathway, therefore research into elucidating the kynurenine pathway has increased.[18,93,94] It has been shown that the kynurenine pathway plays a role in neurodegenerative diseases, tumour proliferation, inflammation and depression.[18,93,94] Kynurenines which include L-kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid and quinolinic acid have been shown to inhibit T-cell proliferation resulting in cell growth arrest of alloreactive T cells and natural killer cells.[93,94] Indolamine 2,3-dioxygenase (IDO) is the first and rate-limiting step in the kynurenine pathway.[93,94] IDO activity in serum can be assessed using the kynurenine-to-tryptophan (Kyn/Trp) ratio, which is the quotient of the first product of the IDO pathway (kynurenine) divided by the substrate tryptophan.[93,94] A lower serum concentration of tryptophan and a higher kynurenine concentration which resulted in a higher Kyn/Trp ratio have been observed in cancer patients.[93,94] Lung cancer patients also showed a high Kyn/Trp ratio which was associated with advanced disease. Furthermore, IDO activity correlates with tumour progression and may facilitate tumour metastasis.[94]

The kynurenine pathway (Figure 5) is initiated by the conversion of L-tryptophan to formyl-kynurenine by the enzyme tryptophan-2, 3-dioxigenase or IDO. Formyl-kynurenine is further broken down to L-kynurenine which has been shown to cross the blood brain barrier where it is further metabolized into several bioactive compounds including kynurenic acid, quinolinic acid, picolinic acid and 3-hydroxyanthranilic acid.[95] The enzymes involved are kynurenine aminotransferase (KAT) which catalyses the breakdown of L-kynurenine into kynurenic acid and kynurenine 3-monooxygenase (KMO) which is responsible for the conversion of L-kynurenine to 3-hydroxykunurenine then to quinolinic acid. Kynurenic acid and quinolinic acid have opposite physiological responses; kynurenic acid

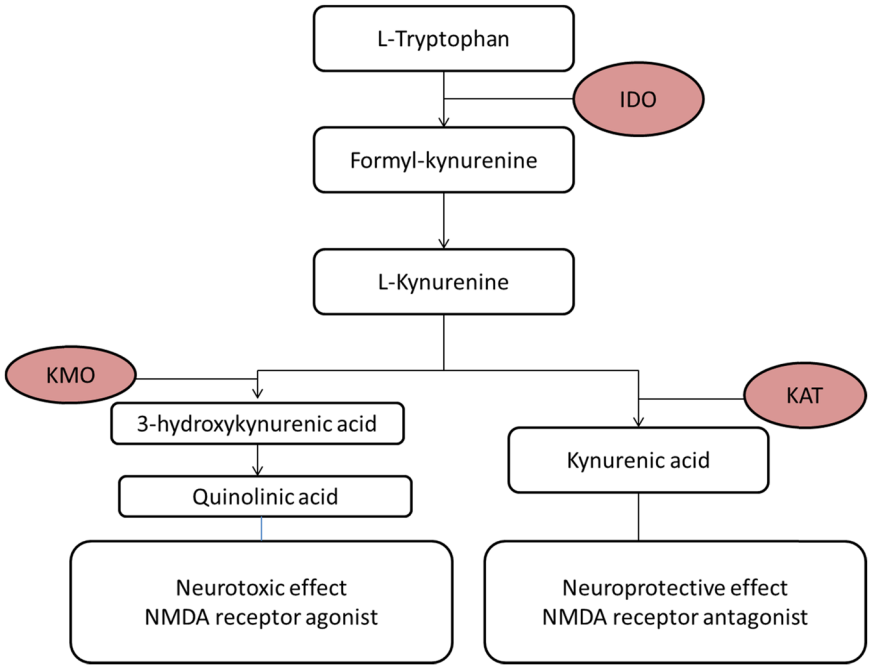

FIGURE 5 The kynurenine pathway. L-Trypophan is metabolized to formyl-kynurenine by the indolamine-2, 3-dioxygenase enzyme. Formyl-kynurenine is further metabolised to L-kynurenine which the precursor of quinolinic acid and kynurenic acid. Quinolinic acid has a neurotoxic effect as a NMDA receptor agonist while kynurenic acid has a neuroprotective effect as an antagonist of the NMDA receptor.[95] (This image was designed using PowerPoint Microsoft 2016 and saved as a tiff image). IDO, indolamine 2,3-dioxygenase; KAT, kynurenine aminotransferase; KMO, kynurenine 3-monooxygenase; NMDA, N-methyl-D-aspartate.

exerts its neuroprotective effect by acting as a N-methyl-D-aspartate (NMDA) receptor antagonist which is a glutamate receptor. Quinolinic acid, however, has a neurotoxic effect by acting as an agonist of the NMDA receptor.[95] An increase in glutamate receptor activity has been shown to cause significant neuronal damage in rats.[95] It has also been reported by Suzuki et al. that an increase in 3-hydroxykynurenine (3HK) which is also a neurotoxic metabolite of kynurenine in mice caused mitochondrial dysfunction through the generation of reactive oxygen species and therefore plays a role in oxidative stress. [93]

10 | KYNURENINE METABOLITES

10.1 | L-Kynurenine

The conversion of L-tryptophan to formyl-kynurenine by the enzyme tryptophan-2,3-dioxigenase or IDO initiates the kynurenine pathway.[94] L-Kynurenine is a metabolite of tryptophan and a direct precursor of kynurenic acid, anthranilic acid and 3-HK.[96] The kynurenine pathway is responsible for 95% of tryptophan degradation in mammals.[96] There are two sources of L-kynurenine in mammals, endogenous sources and exogenous sources. The major endogenous source of L-kynurenine is the liver via the enzyme tryptophan 2,3-dioxigenase (TDO), while IDO activates the synthesis of L-kynurenine during inflammation.[96] L-Kynurenine can also be obtained through the diet (the exogenous source), however the content is low.[97] It has been reported by Werner et al. that various cancer cell lines including hepatoma, colon adenocarcinoma and NB have an increased expression of L-kynurenine as a result of IFN-γ-mediated IDO activation.[96,97]

According to Opitz et al., endogenous production of kynurenine in malignant glioma cells was sufficient to activate aryl hydrocarbon receptors (AhR), which suppressed antitumor immune responses and promoted cancer cell survival and motility.[98] The tryptophan metabolites, L-kynurenine and kynurenic acid are endogenous AhR agonists, which previously displayed inconsistent behaviour in different cells.[99] AhR may thus induce or suppress cell proliferation and survival, depending on the time frame, ligand dosage and category, cell types or whether the experiment was performed in vivo or in vitro.[100]

Tryptophan-derived AhR ligands, including kynurenine, kynurenic acid and FICZ previously displayed antiproliferative and cytotoxic effects in vitro in melanoma A375 and RPMI7951 cells.[7] Importantly, the mechanism of action for each compound differed and was not strictly dependent on AhR.[7] In another study, FICZ inhibited the proliferation of RMA-S murine lymphoma in wild-type immunocompetent mice and B-16 melanoma proliferation in $Rag1^{-/-}$ immunocompromised mice.[99] L-Kynurenine, previously suppressed CXCL12 expression and activity in bone marrow stem cells and thereby also suppressed the autophagy cell survival pathway and induced apoptosis by increasing caspase 3.[8]

10.2 | Kynurenic acid

Kynurenic acid is an endogenous neuroprotectant that is present in the brain at nanomolar concentrations.[101,102] Kynurenic acid is an antagonist to quinolinic acid and it acts on the modulatory site of the NMDA receptor at low concentrations, at higher concentrations, it acts on the glutamate site of the NMDA receptors and on the a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors. Kynurenic acid also

acts as an antagonist to the alpha 7 nicotinic acetylcholine receptors and selectively activates GPR35, a GPCR.[101–104]

Kynurenic acid previously inhibited proliferation in several cancer cell lines and led to the inhibition of ERK 1/2 kinase, the PI3K/Akt pathway, p38 kinases and enhanced β-catenin expression in colon adenocarcinoma (HT-29) cells.[105] Kynurenic acid may exert inhibitory effects on the PI3K/Akt pathway to interfere with proliferation, cell cycle, cell survival and migration; on the p38 pathway to interfere with proliferation, cell motility, apoptosis, stress response and inflammation and on ERK 1/2 kinase in the MAP-K pathway to disrupt cell differentiation and proliferation.[106]

10.3 | Quinolinic acid

Quinolinic acid is a heterocyclic acid that selectively activates the neuronal NMDA subtype of glutamate receptors.[107] Quinolinic acid levels are lower in the brain in comparison the blood and systemic tissues as tryptophan is metabolized to 5-hydroxytryptamine rather than to formylkynurenine.[107] However, during an immune response, IDO-1 activity and quinolinic acid expression increase.[107] During inflammation in the brain, infiltrating macrophages, microglia and dendritic cells are major sources of quinolinic acid production. Astrocytes cannot synthesize quinolinic acid due to absence of the enzyme kynurenine hydroxylase.[107] Astrocytes and neurons are neuroprotective, they uptake quinolinic acid and catabolize it to NAD.[107] Under pathological conditions, the production of quinolinic acid is increased resulting in the toxic accumulation of quinolinic acid within the cells.[107]

11 | CTCE-9908

CTCE-9908 is 17 amino acid (Figure 6), that is analogue to CXCL12 and has been shown to inhibit the interaction of the CXCR4 chemokine receptor with CXCL12. CXCL12/CXCR4 signalling is involved in tumour growth and metastasis. CXCL12/CXCR4 signalling activates actin polymerization and pseudopodia formation subsequently inducing chemotactic and invasive responses at common metastatic sites such as the lungs, bone marrow and lymph nodes.[18,94,101] It inhibits the progression and metastasis of several cancer cells. Treatment of melanoma cells in vitro with 100 µg/ml CTCE-9908 lead to decreased adhesion, decreased cell migration, decreased invasion and a decrease in cell growth. Four-to-six-week-old female Balb/C mice were injected with 106 melanoma cell suspension and then treated with CTCE-9908 (100 µg/ml) for a period of 4 weeks. A 50% decrease in the number of metastatic lung nodules and a decrease in micrometastatic disease were observed.[108] In prostate cancer, CXCR4 expression is upregulated during cancer progression and development.[18] Similarly, Huang and colleagues showed that CTCE-9908 significantly reduced metastasis, as well as primary tumour growth in mouse models of breast cancer.[109] Another study conducted by Kok et al.,[110] the inhibition of CXCR4 activity by CTCE-9908 decreased cell proliferation and induced apoptosis in hepatocellular carcinoma cells (HCC). Phase II clinical trials of CTCE-9908 have been evaluated for patients with HCC. Targeting CXCR4 as a chemotherapeutic treatment can either inhibit the primary tumour growth and metastasis or it can inhibit either tumour growth or metastasis.[109]

CTCE-9908 is the only chemokine receptor antagonist approved by the Food and Drug Administration (FDA) for the treatment of solid tumours especially for the treatment of osteogenic sarcoma.[95,111] Wong et al. showed that CTCE 9908 inhibited the binding of the CXCR4 receptor to the CXCL12 ligand and lead to the reduction in the chemoinvasion of prostate cancer (PC-3) cells.[112,113] They also showed that mice treated with 25 mg CTCE-9908/kg for 4 weeks experienced a reduced metastatic burden.[112,113] Quantification of site-specific metastases showed a 40% reduction in lymph node metastases, spleen metastases were decreased by 75%, a 93% decrease in liver metastases and a 95% reduction in distant metastases was observed in CTCTE-9908-treated mice.[112] The effect of CTCE-9908 on tumour angiogenesis was investigated in primary tumour tissue and it was observed that the tumour samples treated with CTCE-9908 had fewer blood vessels and the vessels were smaller in size.[113] Quantification of microvessel density revealed a decrease in CD34-positive vessel density in the CTCE-9908-treated tumours.[112]

12 | FDA-APPROVED CXC INHIBITORS

Blocking the CXCL12/CXCR4 chemokine pathway is a valid strategy to target various metastatic parameters.[34] Current treatment strategies for melanoma include surgery, chemotherapy, targeted therapies and immunotherapies.[114] However, current melanoma therapy have several limitations, including adverse effects, due to immune

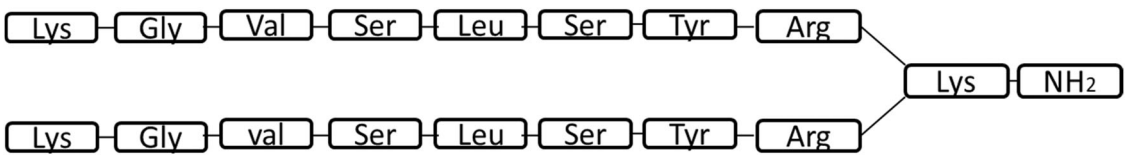

FIGURE 6 Chemical structure of CTCE-9908, a 17 amino acids CXCR4 inhibitor. CTCE-9908 is a modified peptide antagonist for CXCL12 corresponding to the N-terminal region of the CXCL12 chemokine.[18] (This image was designed using PowerPoint Microsoft 2016 and saved as a tiff image). CXCL12, CXC chemokine ligand 12; CXCR4, CXC chemokine receptor 4.

reactions and lack of tumour cell specificity, as well as reduced efficiency due to drug resistance.[16] However some inhibitors showed promising results and were therefore approved by the FDA, such as Maraviroc, an inhibitor of C-C chemokine receptor 5 (CCR5) for the prevention of HIV infection. CCR5 is a seven-transmembrane GPCR that binds several endogenous chemokines namely C-C chemokine ligand CCL7, CCL8 and CCL13.[115] Binding of CCR5 to an agonistic chemokine triggers a signalling cascade via intracellular G-coupled proteins located at the C-terminus to coordinate leukocyte trafficking and recruit immune effector cells to damaged tissues, resulting in the adaptive immune response.[115] A second small-molecule drug, a CXCR4 antagonist for the mobilization of hematopoietic stem cells was approved by the FDA in 2008.[115] A phase III clinical trial on an intestine homing molecule, CCR9 inhibitor showed promising results for the treatment of Crohn's disease.[116]

13 | CURRENT AVAILABLE CTCE-9908 COMBINATION TREATMENTS

The anti-osteolytic effect of the combination of CTCE-9908 with an E-selectin inhibitor (GMI-1359) was investigated in in vitro and in vivo models of metastatic castration-resistant prostate cancer (mCRPC).[113] Hassan et al. used a combination of CTCE-9908 with paclitaxel and docetaxel to observe the effects on ovarian and breast cancer.[117] In ovarian cancer, CTCE-9908 in combination with paclitaxel-induced multinucleation, G2/M arrest and abnormal mitosis.[117]

Hassan and colleagues[117] showed that the combination of CTCE-9908 and docetaxel had an antitumour and antimetastatic effect in breast cancer mouse models. The effect of CTCE-9908 and docetaxel on primary breast tumour growth was investigated in a mouse model, it was found that the combination of CTCE-9908 and docetaxel resulted in a 38% decrease in tumour volume and an 83% reduction in the number of metastases when compared to the control.[117]

14 | FUTURE COMBINATION THERAPIES

Due to the extensive survival capabilities of cancer cells, regulated by various overlapping signalling pathways, single-agent targeted cancer therapies, inhibiting a single key cancer-associated pathway may not alleviate a hallmark capability.[118] Although single-agent-targeted cancer therapies have indeed proven to be viable treatments in in vitro experiments, most clinical responses are transient and frequently result in relapse, as cancer constantly adapts to strengthen its resistance against treatment.[118,119] A potential approach to circumvent mechanisms of drug resistance while simultaneously yielding therapeutic anticancer benefits involves the concept of combination therapy,[120] which is described as a treatment strategy involving the combination of two or more therapeutic agents.[120] Combination therapy aims to target various cancer survival pathways simultaneously in a synergistic or additive manner[120] to cotarget multiple hallmark capabilities.[118]

The kynurenine-induced suppression of CXCL12 expression (previously demonstrated in bone marrow stem cells),[8] together with the CTCE-9908-induced suppression of CXCR4 (previously demonstrated in eight different murine cancer models)[121] may considerably downregulate pathways associated with the CXCL12/CXCR4 axis. Moreover, the inhibitory effects of CTCE-9908 on the CXCL12/CXCR4 axis may be enhanced by kynurenic acid, which previously demonstrated inhibition of ERK 1/2 kinase and the PI3K/Akt pathway in colon adenocarcinoma (HT-29) cells.[105] Tryptophan metabolites, 3-hydroxykynurenine and 3-hydroxyanthranilic acid previously exhibited an additive effect in T-cell suppression when mixed with kynurenine, suggesting an increased cytotoxic effect.[122]

15 | CONCLUSION

Various therapeutic approaches have proved to be inefficient against cancer growth and metastasis. Previous research has shown the effect of the CTCE-9908 compound on prostate and breast cancer cells. The CTCE-9908 compound inhibits the binding of CXCL12 ligand to the CXCR4 receptor leading to reduced metastasis. The kynurenine metabolites previously demonstrated cytotoxicity in various cell lines, but its effect on cancer has not been elucidated.

The kynurenine-induced suppression of CXCL12 expression (previously demonstrated in bone marrow stem cells), together with the CTCE-9908-induced suppression of CXCR-4 (previously demonstrated in eight different murine cancer models) may considerably downregulate pathways associated with the CXCR-4/CXCL12 axis. The assessment of CTCE-9908 and kynurenine metabolites, individually and in combination with each other, may contribute to knowledge of cell–cell or cell–ECM interactions and downstream signalling molecules that impact melanoma and NB cell adhesion.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

No additional data are available.

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