Role of chemokine receptors in thyroid cancer and immunotherapy

Francesca Coperchini1, Laura Croce1,2, Michele Marinò3, Luca Chiovato1,4 and Mario Rotondi1,4

1Istituti Clinici Scientifici Maugeri IRCCS, Unit of Internal Medicine and Endocrinology, Laboratory for Endocrine Disruptors, Pavia, Italy
2PHD Course in Experimental Medicine, University of Pavia, Pavia, Italy
3Endocrinology Unit I, Department of Clinical and Experimental Medicine, University Hospital of Pisa, University of Pisa, Pisa, Italy
4Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy

Correspondence should be addressed to L Chiovato: luca.chiovato@icsmaugeri.it

Abstract

Inflammation is currently regarded as an essential component of malignancies. It is now known that the tumor microenvironment may profoundly influence the biological behavior of cancer cells and ultimately the patient’s outcome. Chemokine and their receptor play a major role in determining the immune phenotype of the cells infiltrating the thyroid tumor microenvironment. Experimental evidence shows that both normal and cancer thyroid cells express specific chemokine receptors. The expression of at least some of these receptors exerts several biological effects, which influence the course of the disease. The present review article will take into account the role of the most studied chemokine receptors (CXCR1, CXCR2, CXCR3, CXCR4, CXCR7, DARC, CCR3, CCR6 and CCR7) in the context of thyroid cancer. This review will focus on current knowledge provided by in vitro and in vivo studies specifically performed on thyroid cancer including (i) expression of chemokine receptors in normal and cancer thyroid cells; (ii) role of chemokine receptors in affecting the biological behavior of thyroid tumors including the metastatic process; (iii) current knowledge about immunotherapies through targeting of chemokine receptors in thyroid cancer.

Introduction

The endocrine and the immune systems are characterized by a cross-talk aimed at maintaining homeostasis (Kelley 1988). A share of common ligands (hormones and cytokines) and their specific receptors is responsible for this interaction, which occurs, not only in the context of endocrine autoimmunity, but also during the development of cancer (Kelley 1988). Inflammatory molecules and their receptors are able to influence cancer survival and progression (Balkwill 2003, Balkwill 2012). In particular, the interaction of chemokines and their receptors mediates immune cell trafficking into the tumor microenvironment resulting in the recruitment and activation of different cell types. The characteristics of this immune cell infiltrate determines the balance between anti-tumor and pro-tumor responses (cell growth, angiogenesis and metastasis) (Balkwill 2003, 2012). Differentiated thyroid cancers are the most prevalent endocrine malignancies being in most cases slow growing, clinically indolent lesions with an overall good prognosis and a low-mortality rate (Haugen et al. 2017). The association between chronic inflammation and thyroid cancer has long been recognized (Mantovani et al. 2010, Allavena et al. 2011). Briefly, a mixture of immune cells and soluble mediators, interacting together through binding receptors, is present within or nearby the tumor, and it is considered to play a major role in tumor
progression and clinical outcome (Cunha et al. 2014). Chemokines are soluble mediators, some of which are produced not only by immune cells, but also by normal thyroid cells and by cancer cells within the thyroid tumor microenvironment (Rotondi et al. 2018). Chemokines regulate the traffic and the behavior of cells by binding seven transmembrane spanning G protein-coupled receptors (Chow & Luster 2014). The present review deals with the role of chemokine receptors in thyroid cancer, their contribution in the metastatic processes and recent findings on their role for immunotherapy.

**Chemokine receptors and cancer**

The effects of chemokines on target cells are mediated by specific receptors (Rossi & Zlotnik 2000, Chow & Luster 2014), which are responsible for the activation of a cascade of cellular responses after binding with their respective ligand (Rossi & Zlotnik 2000). Chemokine receptors are composed of seven transmembrane (7TM) domains and are further classified as ‘typical’ and ‘atypical chemokine receptors’. Nineteen of 23 known chemokine receptors are classified as ‘typical chemokine receptors’, because they are G protein-coupled receptors. (Legler & Thelen 2018). The remaining four receptors are classified as ‘atypical chemokine receptors’, because they share the seven transmembrane domain structure of conventional chemokine receptors, but do not couple to G proteins. Consequently, they fail to induce the classical signaling and downstream cellular responses, acting as scavengers targeting chemokines for lysosomal degradation (Legler & Thelen 2018). Because the chemokine/chemokine receptors system is typically redundant, most chemokine receptors bind two or more chemokines and some chemokines bind both typical and atypical receptors (Zlotnik et al. 2006, Ulvmar et al. 2011).

Metastatic spread is the primary cause of morbidity and mortality in cancer patients. Several lines of evidence indicate that the metastatic infiltration of distant organs is mediated by chemokines and by their cognate receptors (Balkwill 2012, Caronni et al. 2016). Strong support to this notion derives from in vitro and in vivo (animal models) studies, which demonstrate that blocking specific chemokine/chemokine receptor axes does reduce the development of metastasis in several cancers. These include breast (Williams et al. 2010), prostate (Darash-Yahana et al. 2004), lung (Su et al. 2005, Speetjens et al. 2009), colorectal (Jung et al. 2017) and gastric (Zhao et al. 2011) cancer, as well as glioblastoma (Terasaki et al. 2011). These data support the so-called ‘homing theory’ based on the concept that different organs produce chemokines, which can attract the correspondent chemokine receptor-bearing cancers cells (Fig. 1) (Homey et al. 2002, Zlotnik et al. 2011).

**Thyroid cancer and chemokine receptors**

Literature data show that several chemokine receptors play a role in thyroid cancer; these include CXCR1, CXCR2, CXCR3, CXCR4, CXCR7, DARC, CCR3, CCR6 and CCR7 (Fig. 2). Table 1 summarizes our current knowledge on the role of these receptors in thyroid cancer.

**CXCR1 and CXCR2**

The CXC chemokine receptors 1 and 2 are generally expressed simultaneously. CXCR1 is the receptor for CXCL1, CXCL6, CXCL7 and CXCL8 chemokines (Rotondi et al. 2007, Waugh & Wilson 2008). CXCR1 is mainly expressed in neutrophils and was originally characterized by its ability to induce the chemotaxis of leukocytes. CXCR1, originally shown to act on multiple cell types, is involved in invasion and migration of estrogen receptor (ER)-negative breast cancer cells (Holmes et al. 1991, 2009, Jiang et al. 2013). In several solid tumors, the expression of CXCR1 is positively correlated with drug resistance, cell invasion and metastasis (Jiang et al. 2013). CXCR2 binds the CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7 and CXCL8 chemokines. Among them, CXCL8 plays a major role in thyroid cancer microenvironment (Rotondi et al. 2007, 2018). CXCR2 was described originally in leukocytes and subsequently in non-hematopoietic cells, such as keratinocytes, neurons and epithelial cells, in regions corresponding to intermediate levels of differentiation (Baggiolini et al. 1989, Tecimer et al. 2000, Waugh & Wilson 2008). Immunohistochemical analysis of CXCR2 expression revealed that it is universally present in cells of the dispersed neuroendocrine system (cells in the central and peripheral nervous systems) and in squamous epithelial cells (Tecimer et al. 2000, Waugh & Wilson 2008). The first study investigating these receptors in thyroid cells demonstrated their expression in monocytes, granulocytes and infiltrating cells of thyroid cancer and in para-follicular C cells (Aust et al. 2001). In 2015, Zhao et al., demonstrated, by immunohistochemistry, the expression of CXCR1 protein in 70% of 106 follicular thyroid cancers cells (FTCs) and in 27.3% of 128 follicular thyroid adenomas (FTAs) (Zhao et al. 2015).
The role of CXCR1 and CXCR2 in promoting metastatic thyroid cancer is still debated. Some but not all studies found a cancer-promoting effect. In 2014, Tang et al. analyzed by immunohistochemistry the expression of CXCR1 in 129 papillary thyroid cancers (PTCs), 61 hyperplastic thyroid nodules and 118 normal thyroid tissue specimens (Tang et al. 2014). They found the CXCR1 molecule to be highly expressed in PTCs and significantly correlated with lymph node metastases (Tang et al. 2014). Real-time RT-PCR showed an upregulation of the CXCR1 mRNA in PTCs which significantly correlated with lymph node metastases (Tang et al. 2014). These results prompted subsequent studies aimed at evaluating the expression of CXCR1 and CXCR2 in thyroid cancer cells and their involvement in the metastatic process. Fang et al. evaluated the expression of CXCR1 and CXCR2 in normal and neoplastic thyroid cells, the latter both in surgical specimens of PTC and in PTC cell lines (Fang et al. 2014). Immunostaining for CXCR1 and CXCR2 was performed in 26 PTCs and in 6 normal thyroid tissue specimens. Eighty-three percent of PTC specimens showed a strong staining for CXCR1; the corresponding figure for CXCR2 was 71.5%. Virtually no staining occurred in normal thyroid tissue specimens. A positive cell membrane staining for both CXCR1 and CXCR2 was also detected in three PTC cell lines: K1, TPC-1 and BCPAP (Fang et al. 2014). At variance with the previous study by Tang et al., no association was found between the expression of CXCR1 and CXCR2 and the occurrence of lymph node metastasis and/or of extra-thyroid extension (Fang et al. 2014). In a further study, Visciano et al. (2015) confirmed the observations by Tang et al. (2014),

Figure 1
Representation of the homing theory: cancer cells expressing a given chemokine receptor metastatize from the cancerous mass to distinct organs being attracted by the respective binding chemokine gradient. A full colour version of this figure is available at https://doi.org/10.1530/ERC-19-0163.
reporting that CXCR1 and CXCR2 were expressed in both normal and thyroid cancer cells. The negative finding of Fang in normal thyroid cells was probably due to the rather low number of normal thyroid tissue specimens (6). In a recent study, Cui et al. investigated the involvement of the CXCL5–CXCR2 axis in epithelial-to-mesenchymal transition (EMT) of PTC (Cui et al. 2018). The activation of this chemokine receptor axis promoted the migration and invasiveness of PTC cells in vitro (Cui et al. 2018). The expression of EMT-associated molecules E-cadherin, N-cadherin and vimentin was upregulated in response to recombinant human-(rh)-CXCL5 stimulation. As assessed by Western blot analysis, treatment with rh-CXCL5 upregulated the markers of EMT, while, in knocked down cells for CXCR2, a downregulation occurred (Cui et al. 2018). These findings suggest that the interaction of CXCL5 with CXCR2 has a role in the EMT process of thyroid cancer.

CXCR3

The CXC chemokine receptor 3 is commonly associated with Th1 immune responses and Th1-associated diseases. CXCR3 is the receptor for Th1-type chemokines (CXCL9, CXCL10 and CXCL11), which are strongly upregulated by IFN-γ stimulation (Loetscher et al. 1996, Cole et al. 1998, Rotondi et al. 2007) and for CXCL4. After IFN-γ stimulation, thyrocytes secrete CXCR3-binding chemokines, which in turn recruit Th1 lymphocytes-expressing CXCR3 and secreting IFN-γ. This sequence of events strongly supports the concept that IFN-γ inducible chemokines and their receptor CXCR3 play an important role in the initiation of autoimmune thyroiditis (AITD) (Rotondi et al. 2003, 2007). Subsequently, it was demonstrated that the binding of CXCR3 by its chemokine ligands drives the traffic of Th1 cells, CD8+ T cells and NK cells into the tumor microenvironment (Nagarsheth et al. 2017). CXCR3 expression is upregulated in many tumors, suggesting its pro-tumorigenic role in the inflammatory microenvironment (Abron et al. 2018, Susek et al. 2018). In this scenario, we know that different CXCR3 ligands (CXCL4, CXCL9, CXCL10 and CXCL11) can trigger different and sometimes opposite responses. This is, at least in part, due to the presence of two splicing variants of the receptor: CXCR3A involved in cell proliferation and CXCR3B involved in cell apoptosis (Urra et al. 2018).
The first demonstration that CXCR3 is also involved in cancer was provided by Tsuchiya et al. in 2004. These authors investigated the expression of CXCR3 in different subtypes of peripheral-T-cell-lymphoma (PTCL), reporting that the expression of CXCR3 was associated with a better prognosis as assessed by a longer survival (Tsuchiya et al. 2004).

Similar findings were obtained in two rare cases of thyroid PTCL, complicating chronic autoimmune thyroiditis (Koida et al. 2007).

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Table 1  Chemokine receptors expression and role in thyroid cancer.

<table>
<thead>
<tr>
<th>Chemokine receptor</th>
<th>Binding chemokine/s</th>
<th>Thyroid cells expression</th>
<th>Infiltrating immune cells expression</th>
<th>Relevance for thyroid cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCR1</td>
<td>CXCL1, CXCL6, CXCL7 and CXCL8</td>
<td>Normal human thyroid cells (Tang et al. 2014, Visciano et al. 2015) Rat PTC (Fang et al. 2014) Human FTC, FTA (Tang et al. 2014, Zhao et al. 2015)</td>
<td>TAM (Fang et al. 2014)</td>
<td>Association with human lymph node metastasis (Tang et al. 2014) No association with lymph node metastasis or extra-thyroid extension in rats (Fang et al. 2014)</td>
</tr>
<tr>
<td>CXCR2</td>
<td>CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8</td>
<td>Rat PTC (Fang et al. 2014)</td>
<td>TAM (Fang et al. 2014)</td>
<td>Promotion of EMT in vitro (Cui et al. 2018) No association with lymph node metastasis or extra-thyroid extension in rats (Fang et al. 2014)</td>
</tr>
<tr>
<td>CXCR3</td>
<td>CXCL4, CXCL9-10-11</td>
<td>Normal human thyroid cells, human TPC-1 cell line, non-metastatic human PTC (Urra et al. 2018)</td>
<td>No data available</td>
<td>No data available</td>
</tr>
<tr>
<td>CXCR7</td>
<td>CXCL12 and I-TAC</td>
<td>Human PTC (Liu et al. 2014)</td>
<td>No data available</td>
<td>Association with lymph node metastasis (Liu et al. 2014)</td>
</tr>
<tr>
<td>DARC</td>
<td>CXCL8, CXCL5, CXCL6, CXCL11, CCL2, CCL5, CCL7, CCL11, CCL14, CCL17</td>
<td>Normal human thyroid cells, human FTA, human FTC, human CVPTC, human FVPTC (Latini et al. 2013)</td>
<td>No data available</td>
<td>No data available</td>
</tr>
<tr>
<td>CCR3</td>
<td>CCL4, CCL5, CCL7, CCL11, CCL13, CCL15, CCL24, CCL26, CCL28</td>
<td>Normal human thyroid tissue, human thyroid cancer tissue (González et al. 2009, Tang et al. 2018)</td>
<td>No data available</td>
<td>No data available</td>
</tr>
<tr>
<td>CCR7</td>
<td>CCL19 and CCL21</td>
<td>Human PTC, MTC (García-López et al. 2001), primary cultures of papillary human thyroid cancer cells with RET/PTC rearrangement expression (Zhang et al. 2016)</td>
<td>Lymphocyte and dendritic cell (Sancho et al. 2006)</td>
<td>Association with human lymph node metastases (Zhang et al. 2016)</td>
</tr>
</tbody>
</table>

ATC, anaplastic thyroid cancer; CVPTC, classical variant of papillary thyroid cancer; EMT, epithelial-to-mesenchymal transition; FTA, follicular thyroid adenoma; FTC, follicular thyroid cancer; FVPTC, follicular variant of papillary thyroid cancer; MTC, medullary thyroid cancer; PTC, papillary thyroid cancer; TAM, tumor-associated macrophages.
The common belief that the major role of the CXCR3 chemokine receptor lies in thyroid autoimmune disease rather than in thyroid cancer development is probably the reason for the lack of systematic data on this issue. The topic was addressed again in 2016, when Jiskra et al. investigated the expression of some chemokine receptors in lymphocytes from benign and malignant thyroid nodules derived from patients with and without Hashimoto’s thyroiditis (Jiskra et al. 2016). Flow cytometry analysis showed that lymphocytes from neoplastic nodules had a higher expression of CXCR3 compared with those from the benign ones (Jiskra et al. 2016). More recently, Urra et al. characterized the expression pattern of the CXCR3 variants (CXCR3A and CXCR3B) in benign tumors and PTC (Urra et al. 2018). Interestingly, these two CXCR3 splicing variants have profoundly different function, because CXCR3A promotes cell viability, proliferation and angiogenesis, while CXCR3B displays angio-static and apoptotic effects (Lasagni et al. 2003).

Immunohistochemistry analysis revealed that total CXCR3 levels were increased in PTC compared with normal contralateral tissue. CXCR3A and CXCR3B levels were also evaluated in non-metastatic PTC and metastatic PTC. The results showed that upregulation of CXCR3A and CXCR3B was associated with absence of lymph node metastasis in PTC, but CXCR3B protein was higher in metastatic PTC. (Urra et al. 2018).

**CXCR4**

The CXC chemokine receptor 4 binds CXCL12. The role of the CXCL12/CXCR4 signal was originally postulated in the fields of immunology, of hematopoiesis, of lymphocyte homing and of HIV infection (Brelot et al. 1997, Zou et al. 1998, Franitza et al. 2002). CXCR4 is by no doubts the most studied chemokine receptor in thyroid cancer. CXCR4 is not expressed by normal thyroid cells (Castellone et al. 2004). CXCR4 was first detected in a rat thyroid cell line characterized by the presence of a RET/PTC rearrangement being the level of expression associated with the cell ability to invade and migrate. In addition, CXCR4 also displayed a relevant anti-apoptotic effect on cancer cells (Castellone et al. 2004). Further confirmation of the pro-tumorigenic effect of CXCR4 derived from experiments showing that treatment with a CXCR4-blocking antibody abolished thyroid cancer cell migration induced by treatment with CXCL12 (Castellone et al. 2004). CXCR4 was also shown to be expressed in human neoplastic cell lines derived from PTC and in anaplastic thyroid cancer (ATC) (Hwang et al. 2003, De Falco et al. 2007). Also in these cell lines, treatment with CXCL12 induced cell proliferation and motility which were reverted by the CXCR4 antagonist AMD3100 or by interfering with the CXCR4 mRNA synthesis (De Falco et al. 2007). Immunohistochemical analysis aimed at detecting CXCR4 in thyroid lesions showed that this receptor is mainly distributed in the nucleus of thyroid cancer cells, with weak staining in the cytoplasm. No expression was detected in the adjacent benign tissue. The CXCR4 staining pattern was stronger in more aggressive thyroid carcinomas, such as poorly differentiated thyroid carcinoma (100%) and medullary thyroid carcinoma (85.7%). A lower expression of CXCR4 was found in the less aggressive variants, such as classic PTC (68.8%) and follicular thyroid carcinoma (66.7%). Shin et al. reported a different immunohistochemical pattern of CXCR4 according to the histologic subtype of PTC. Indeed, CXCR4 expression was greater in poorly differentiated (81.0%) than in classic PTC (50.0%); low expression levels were also found in follicular (33.9%) and in the diffuse sclerosing variants (14.3%) of PTC (Shin et al. 2013). Although at strikingly lower levels, CXCR4 is also expressed in benign conditions, including Hashimoto thyroiditis (15.8%), follicular adenoma (8.0%) and nodular goiter (6.7%) (He et al. 2010). It seems worth highlighting that the expression of CXCR4 also influences cell traffic in thyroid cancer microenvironment. Indeed, in anaplastic thyroid cancer (ATC) a significant correlation between CXCR4 expression and numbers of tumor-associated macrophages (TAMs) was reported (Kim et al. 2016). CXCL12 induced migration in human thyroid anaplastic carcinoma cell lines (ARO), which was inhibited by an anti-CXCR4 antibody (Hwang et al. 2003). A further confirmation of the role of CXCR4 in thyroid cancer aggressiveness and metastatic behavior stems from the study of Gonzalez et al. By using immunohistochemistry and flow cytometry analysis, these authors demonstrated that CXCR4 is found in nearly 90% of PTC cells, being its expression correlated with node metastases. The expression of CXCR4 was also found to be positively correlated with tumor size in PTC (Werner et al. 2017). Taken together, these findings support the concept that the expression of CXCR4 is positively related to more aggressive tumors and/or to those with a poorer prognosis (González et al. 2009).
Based on the above described preclinical data, the expression of CXCR4 was searched for in thyroid cancer cells within the preoperative diagnostic work-up of thyroid lesions. In a retrospective study, Torregrossa et al. evaluated the expression of several markers including CXCR4 in thyroid fine-needle aspiration cytologic (FNAC) specimens obtained from 100 patients who had undergone thyroidectomy for a solitary nodule (Torregrossa et al. 2010). Results of FNAC and immunohistochemistry analysis were related to the final histological diagnosis. They found that CXCR4 was overexpressed in the vast majority of PTCs (92%). On the other hand, taking into account all the lesions classified as benign (by cytology and post-surgery histology), 96% were negative for CXCR4 (Torregrossa et al. 2010). In addition, the overexpression of CXCR4 was linked to a more aggressive thyroid cancer phenotype (Torregrossa et al. 2012). CXCR4 is also involved in the metastatic process of thyroid cancer. By using RT-PCR, Wang et al. demonstrated that the levels of TWIST (a marker of EMT) and of the mRNA for CXCR4 were significantly greater in PTCs than in normal thyroid tissues and that the levels of both the protein and the mRNA for CXCR4 were correlated with the presence of metastasis (Wang et al. 2013).

**CXCR7**

The CXC chemokine receptor 7 mediates cellular adhesion, migration, proliferation and survival by binding CXCL12 and CXCL11 (Burns et al. 2006, Boldajipour et al. 2008). CXCR7 was found to be expressed by 65.8% of PTC being its level of expression correlated with lymph node metastasis (Liu et al. 2012). Subsequently, the same group demonstrated that CXCR7 is markedly expressed in PTC, both at the mRNA and the protein level, this expression being positively related with tumor progression (Liu et al. 2014). It was also shown that knockdown of CXCR7 in PTC cells suppressed their proliferation and invasiveness, decreased the expression of some promoting factor for cell growth (cyclin A, CDK2 and PCNA) and increased the expression of two cell-cycle inhibitors (p21 and p57). These changes resulted in S phase arrest and promoted apoptosis. Taken together, these findings pointed toward an important role of this receptor in thyroid cancer metastasis (Liu et al. 2014).

The mechanisms involved in the effect of CXCR7 on tumor progression were studied by Zhang et al. who showed that CXCR7 regulates growth and metastasis of PTC cells via the activation of the PI3K/AKT pathway and its downstream NF-κB signaling, as well as via the downregulation of cell differentiation, by downregulating Notch signaling (Zhang et al. 2015). Furthermore, Zhang et al. (2016) recently reported that a high expression of CXCR7 increases both CXCL8 and VEGF levels, two main molecules with proven pro-tumorigenic effect (Rotondi et al. 2018).

**The CXCR7/CXCR4/CXCL12 axis**

In the previous paragraphs, we focused on the expression of a single chemokine receptor in thyroid cancer cells and its relation with cancer progression and development. However, some studies focused on the role of a peculiar chemokine receptors combination (CXCL12/CXCR4/CXCR7) in driving cancer progression and metastasis in human cancer (Sun et al. 2010). Both in vitro and in vivo studies demonstrated that the role of the CXCL12/CXCR4 axis in cancer progression is mainly to facilitate metastasis and mobilization of cancer cells (Sun et al. 2010). Zhou et al. found that the expression of CXCR4, CXCL12 and CXCR7 varied in malignant and benign thyroid tissue specimens and that the overexpression of CXCR4 and CXCR7 enhanced thyroid cancer cell invasiveness, but not their proliferation (Zhu et al. 2016). The role of CXCL12/CXCR4/CXCR7 was also investigated in medullary thyroid cancer (MTC) cells where it was found to influence the biological behavior of neoplastic cells, likely through a regulation of the EMT process (Werner et al. 2017). The CXCR4/CXCR7/CXCL12 axis has a role also in FTC. Werner et al. examined CXCR4/CXCR7 expression in 44 FTCs and in the corresponding non-neoplastic thyroid tissue, in 10 distant metastases of FTC and in 18 follicular adenomas. They observed that expression of CXCR4/7 was associated with larger tumor size, advanced stage at diagnosis and shorter overall and recurrence-free survival. Interestingly, CXCR4 expression was significantly greater in distant metastases as compared with the primary tumor. Again, CXCL12 induced cell growth, cell-cycle activation and EMT, while CXCR4 antagonists significantly reduced FTC invasiveness in vitro (Werner et al. 2018).

**CCR3**

CC-chemokine receptor 3, a member of the CC receptor family, binds several chemokines: CCL4, CCL5, CCL7, CCL11, CCL13, CCL15, CCL24, CCL26, CCL28 (Vela et al. 2015). CCR3 enhances cellular proliferation, invasion and migration, mainly through the regulation of ERK and JNK signaling pathways, in different cancer cell types.
CCR3 was first identified in renal cell carcinoma (Jöhner et al. 2005). Only two studies evaluated its expression in thyroid cancer (González et al. 2009). Gonzales et al. showed that the expression of CCR3 was higher in PTC by immunohistochemistry (2.5-fold versus normal tissue) and by flow cytometry (3.5-fold versus normal thyroid cells). However, CCR3 was similarly expressed in tumor cells from patients with or without lymph node metastasis. Similar results were recently reported by Tang et al. using a bioinformatic analysis method (Tang et al. 2018). These findings do not support a prognostic role of CCR3 expression in thyroid cancer (González et al. 2009).

CCR6

CC-chemokine receptor 6 is the sole receptor for the chemokine CCL20. The expression of CCL20 and CCR6 is increased in many cancers such as colorectal and pancreatic cancers being associated with a poor prognosis (Rubie et al. 2010, Frick et al. 2013). In thyroid cancer, a role of CCR6 was suggested by the observation that dendritic cells expressing CCR6 are found to be densely accumulated in PTC (Tsuge et al. 2005). In a mouse model of liver metastasis, Dellacasagrande et al. demonstrated that CCR6 is overexpressed in metastatic cells originating from colorectal, thyroid and ovarian cancers. This finding would fit with the notion that human liver cells constitutively secrete CCL20, which in turn recruits those cancer cells expressing CCR6 (Dellacasagrande et al. 2003). More recently, Zeng et al. showed that thyroid cancer cell lines (TPC-1, BCPAP, FTC-133 and SW1736 cells) and normal thyrocytes (HTori-3) express the CCR6 mRNA and produce the relative protein (Zeng et al. 2014). Further studies demonstrated that incubation with CCL20 induces invasiveness and migration of these thyroid cancer cell lines, a phenomenon promptly reverted by knocking down CCR6. These experimental results support the concept that the CCL20–CCR6 axis mediates invasiveness and metastatization of thyroid cancer cells. Moving from these data, our group showed by immunocytochemistry and flow cytometry that the basal expression of CCR6 is significantly upregulated by TNF-α in two thyroid cancer cell lines: TPC-1 and BCPAP (Coperchini et al. 2016).

CCR7

The CC-chemokine receptor 7 is expressed on activated T and B lymphocytes as well as on dendritic cells, being strongly upregulated in B cells infected with Epstein–Barr virus and in T cells infected with herpes virus (Campbell et al. 1998, Yanagihara et al. 1998). CCR7 is the chemokine receptor for CCL19 and CCL21 (Nagarsheth et al. 2017). CCR7 plays a critical role in lymphocyte and dendritic cell trafficking into and within lymph nodes (i.e. the preferential metastatic site for PTC and MTC) (Sancho et al. 2006). Sancho et al., reported that, as evaluated by RT-PCR, the expression of CCR7 was higher in PTC and MTCs than in follicular and poorly differentiated thyroid carcinomas, being positively correlated with lymph node metastases (Sancho et al. 2006). Incubation with CCL21 also promoted proliferation and migration of the CCR7-expressing thyroid tumor cell line TPC-1 (Sancho et al. 2006). Although from a quantitative point of view, CCR7 is scarcely detected in PTC cells (5–10%) being absent in normal thyroid cells (González et al. 2009), its expression, both at the mRNA and at the protein level, was found to be associated with larger tumor size and increased markers of tumor aggressiveness in PTC (Wagner et al. 2008). Proliferation assays in primary cultures of PTC cells indicated that CCR7 activation by CCL21 is associated with a significant increase of cell proliferation (Zhang et al. 2016). Flow cytometry analysis indicated that CCL21/CCR7 interaction significantly increased the fraction of cells in the G2/M phase of the cell cycle. Western blotting experiments confirmed that CCL21/CCR7 interaction significantly upregulated cyclin A, cyclin B1 and cyclin-dependent kinase 1 (CDK1) expression (Zhang et al. 2016).

The atypical receptor DARC

Duffy, also named DARC (Duffy antigen receptor for chemokines), is a glycosylated membrane protein that belongs to the subfamily of silent chemokine receptors, which selectively binds angiogenic chemokines, such as CXCL8 (but also CXCL5, CXCL6, CXCL11, CCL2, CCL5, CCL7, CCL11, CCL14, CCL17) (Latini et al. 2013). The expression of DARC was found to be elevated in patients with breast cancer. The relationship between DARC and thyroid cancer was evaluated in a single study by Latini et al. (2013). By using quantitative PCR, DARC expression was searched for in 18 normal thyroid tissues, in 15 FTA and in 139 malignant thyroid tumors. DARC expression was higher in benign lesions compared with the malignant ones. Moreover, DARC expression was significantly lower in those tumors with a greater than 10 mm diameter (Latini et al. 2013). When different subtypes of thyroid...
carcinoma were compared, similar expression levels of DARC were observed (Latini et al. 2013). DARC expression was also significantly reduced in PTCs associated with lymphocyte infiltration when compared with normal thyroid tissue. (Latini et al. 2013), suggesting an inverse relationship between DARC expression and thyroid cancer aggressiveness.

**Chemokine receptors and immunotherapy**

Effective strategies for immunotherapy are based on the generation of immunity against tumor-specific antigenic peptides. Given that chemokines and their receptors have crucial roles in inflammatory human diseases, efforts have been made to target chemokine networks in patients with autoimmune diseases and chronic inflammation (Nagarsheth et al. 2017). As crucial mediators of cell migration, chemokines are also an interesting target for therapies aimed at attenuating inflammation without inducing generalized immunosuppression in several clinical settings, such as cancer, autoimmune disease and allograph transplant (Rot & von Andrian 2004, Rotondi et al. 2007, Rotondi & Chiovato 2011). The most successful example of immunotherapy directed to a chemokine receptor is the development of drugs targeting the CXC chemokine receptor 4 (CXCR4) for hematopoietic stem cell mobilization in patients who require stem cells auto-transplant. Another example is targeting the CC-chemokine receptor 5 (CCR5) in order to inhibit the entrance of HIV in T cells through CCRS binding (Johnson et al. 2005, Gullick et al. 2008, Rotondi & Chiovato 2011).

Targeting chemokine receptors turned out to be a good approach also in several models of cancer (Johnson et al. 2005). Interfering with the chemokine system involves several strategies including antibodies, small molecules, modified chemokines, neutralizing monoclonal antibodies, binding proteins or pharmacological modulation of chemokine/chemokine receptor interactions (Proudfoot et al. 2003, Johnson et al. 2005). These approaches were recently tested in different types of cancer with success at least in some cases. Targeting of the CCL2, CCL3 or CCL5 signaling inhibits metastasis and angiogenesis in mouse models of breast, lung and ovarian cancer (Qian et al. 2011, Long et al. 2012, Bonapace et al. 2014, Kitamura et al. 2015). The blockade of CXCR4–CXCL12 signaling reduced tumor angiogenesis, invasiveness and tumor-induced immunosuppression. Indeed, anti-CXCR4 and anti-CXCL12 antibodies prevented metastasis, reduced tumor weight and prevented tumor extravasation in preclinical models (Bertolini et al. 2002, Lapteva et al. 2005, Zlotnik 2006). Another good example of immunotherapy through chemokine receptor blockade is provided by the targeting of the CXCL8-CXCR1/2 axis, which plays an important role in tumor progression and metastasis by regulating cancer stem cell proliferation and self-renewal (Ha et al. 2017). During the past two decades, several CXCR1/2 small-molecule inhibitors, CXCL8 release inhibitors and neutralizing antibodies against CXCL8 and CXCR1/2 have been developed. Several preclinical studies suggest that combination of CXCR1/2 inhibitors along with other targeted therapies, chemotherapies and immunotherapy may be effective in treating specific types of cancer (Ha et al. 2017).

Two chemokine receptor-targeted agents, which were previously used for stem cells mobilization in stem cells auto-transplant, are currently studied in humans for their anti-cancer properties. Reparixin, an allosteric inhibitor of CXCR1 and CXCR2, was demonstrated to be safe and tolerable in a Phase Ib pilot study in breast cancer patients in association with paclitaxel (Schott et al. 2017). The promising oncological response rate lead to the initiation of a Phase II clinical trial on metastatic triple-negative breast cancer NCT02370238. Plerixafor, an anti-CXCR4 agent currently approved for hematopoietic stem cells mobilization in combination with granulocyte colony-stimulating factor, is object of a still ongoing Phase I study in advanced ovarian, pancreatic and colorectal cancer (EUDRACT 2014-000117-31).

Therapies aimed at targeting chemokine receptors can affect the trafficking of different immune cell subsets and can modify the biological activity of non-immune cells in tumor microenvironment (Nagarsheth et al. 2017). Both direct and indirect manipulations of chemokine–chemokine receptor signaling pathways could provide antitumoral effects by reshaping its immune and biological phenotypes. Although promising results are present, a possible pitfall of immunotherapy directed to chemokine receptors could be due to the redundancy of the chemokine–chemokine receptor system. On the other hand, it could as well be possible that combination therapy with other immune-modulating drugs could lead to better results in cancer therapy.
Table 2  Chemokine receptors targeting for immunotherapy of thyroid cancer.

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<tr>
<th>Compound</th>
<th>Pharmacological effect</th>
<th>In vivo and in vitro effects in thyroid cancer</th>
<th>Reference</th>
</tr>
</thead>
</table>
| REPARIXIN      | Noncompetitive allosteric inhibitor of CXCR1 and CXCR2 | • Dose-dependent inhibition of CAL62 and 8505C cell growth and induction of apoptosis, with no effect on non-cancerous cell lines  
• Inhibition of cell-cycle progression with accumulation in G1 phases of 8505C and SW1736 cells  
• Inhibition of DNA synthesis in cancerous cell lines  
• Reduction of levels of mRNAs encoding for EMT transcription factors (SNAIL1/SNAIL, SNAIL2/SLUG, TWIST1, ZEB1)  
• Reduction of migration of cancerous cell lines  
• Reduction of stemness markers expression  
• Reduction of sphere formation  
• Reduction of xenografted thyroid tumor in nude mice  
• Inhibition of migration and invasion of papillary thyroid cancer cell line (BHP10-3) at low doses  
• Inhibition of CXCL12-induced cell proliferation and motility in PTC and ATC cell  
• Inhibition of BHP10-3M cell proliferation at high doses  
• Inhibition of formation of xenograft tumors in BALB/c mice | Liotti et al. (2017a,b) |
| DF1681Y        |                                               |                                                                                                               |                         |
| PLERIXAFOR     | Antagonist of CXCR4                           |                                                                                                               |                         |
| AMD3100        |                                               |                                                                                                               |                         |

Chemokine receptors and immunotherapy in thyroid cancer

Although most patients respond well to current therapy, 10–30% of thyroid cancer patients eventually develop recurrent disease and tumor metastasis (Ward 2014). Immunotherapy aimed at targeting chemokine receptors for thyroid cancer involves three receptors: CXCR1, CXCR2 and CXCR4 (Table 2). Reparixin, an allosteric, noncompetitive, low-molecular-weight CXCR1–CXCR2 dual inhibitor, was evaluated for a possible inhibition of thyroid cancer viability and stemness. To this purpose, experiments were conducted in the 8505C thyroid cancer cell line, which expresses CXCR1 and CXCR2, and in 8505c cells not expressing CXCR1 and CXCR2 after silencing with small-interfering RNAs (shCXCR1 and shCXCR2) (Liotti et al. 2017b). The percentage of 8505c apoptotic cells was increased by treatment with Reparixin, and completely abolished in cells silenced for CXCR1 or CXCR2. Moreover, treatment with Reparixin significantly reduced stemness and EMT marker expression in 8505C thyroid cancer cells, but not in the same cell line silenced for CXCR1 and CXCR2. It is interesting to note that the ability of 8505c cells to form spheroids was significantly reduced in shCXCR1 and to a lesser extent also in shCXCR2 (Liotti et al. 2017a,b). Taken together, these findings demonstrated the ability of Reparixin to inhibit both CXCR1 and CXCR2 expression which, in turn, produces a reduction of viability and an inhibition of stemness in thyroid cancer cells (Liotti et al. 2017b). Interesting results were found not only by targeting these two receptors, but also by modulating the expression of one of their ligands, CXCL8, a chemokine which binds both CXCR1 and CXCR2. By using different compounds, it was demonstrated that the inhibition of CXCL8 secretion in thyroid cancer cells reduces cell migration in in vitro experiments (Rotondi et al. 2013, 2015, 2016, Awwad et al. 2018, Coperchini et al. 2019). Accordingly, thyroid cancer metastasis is reduced and survival is prolonged in animal models (Fang et al. 2014). CXCR4 targeting was also evaluated in thyroid cancer. Jung et al. reported that the antagonist of CXCR4 (AMD3100) had an anti-tumor effect in the PTC cell line (BHP10-3) (Jung et al. 2016). In particular, AMD3100 displayed a dual effect producing an inhibition of thyroid tumor cells migration at low dose and an inhibition of proliferation at high dose (Jung et al. 2016).

Although these results should be regarded as preliminary, it is reasonable to hypothesize that immunotherapy strategies aimed at targeting chemokine receptors in refractory thyroid cancer will be developed in the near future.

Conclusion

The chemokine–chemokine/receptor system is crucially involved in the recruitment and maintenance of immune cells within the tumor microenvironment. More than 50 chemokine receptors have been identified so far. For nine of these receptors, there is evidence for their expression...
and role in thyroid cells and/or infiltrating immune cells within the thyroid cancer microenvironment. Future advances in our understanding the chemokine receptor biology in thyroid cancer are certainly expected. In particular, the up or downregulation of chemokine receptors by other components of the thyroid tumor microenvironment and their biological consequences still remain to be fully elucidated. Chemokine receptors are involved in thyroid cancer cell growth, aggressiveness and metastatization, with clear impact on the clinical course of malignancy. Although large series prospective human studies are required, it could be envisaged that assessment of chemokine receptors (and of their ligands) might serve as useful biomarkers to guide follow-up and treatment of patients with more aggressive thyroid cancer. The above described features have made chemokine receptors an attractive potential drug target. In this view, recent studies started developing immunotherapy strategies aimed at targeting the interaction of these receptors with their ligand chemokines in thyroid cancer.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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