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# Role of Immune System in Kidney Cancer

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Additional information is available at the end of the chapter

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## Abstract

Almost all kidney cancers are associated to immune dysfunction. Among these, renal cell carcinoma (RCC) represents approximately 2% of malignancies that affect adults and for 90–95% of all kidney cancers. Recent evidences have collaborated to elucidate the mechanisms involved in the development of this disease. In this view, dysfunctional neutrophil migration, as well as T lymphocyte-DC (dendritic cell) cross talk, DC maturation, immune cell metabolism, and reactivity and abnormal expression of cytokines and chemokines and their receptors have been highlighted in RCC and stroma cells. A rational development of novel therapies to recover antitumor activity of immune system is closely related to the understanding of the complex interactions between immune system and tumor. Some insights have been reached and immunomodulatory molecules, such as interleukin-2 (IL-2) and IFN- $\alpha$ , immune checkpoint inhibitors, and chemokines antagonists have shown clinical efficacy. In this chapter, we overview the essential role of innate and adaptive immune response in RCC and discuss drugs approved or in development for its treatment.

**Keywords:** renal cell carcinoma, cytokines, chemokines, natural immunity, adaptive immunity

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## 1. Introduction

Basically, the kidney is assigned to the urine production function. Indeed, kidney function overcomes this definition, since it is critical for the regulation of the body's electrolytes, body's fluid balance, body's acid-base balance, and depuration of body waste. Thereby, diseases such as kidney cancer compromise the kidney's ability to perform its functions and bring consequences to the whole organism.

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According to the cell type where the tumor starts, kidney cancers are classified as renal cell carcinoma (RCC) subdivided in papillary RCC, chromophobe RCC, rare types of RCC, and unclassified RCC. Also, there are transitional cell carcinoma, Wilms' tumor, which almost always occur in children and renal sarcoma [1–4].

Regardless of the category, kidney tumors have been associated with immune dysfunction [1–3, 5]. RCCs are rich in immune infiltrates consisting of T cells, natural killer (NK), DCs, macrophages among others [6, 7]. Different functions are ascribed to the different subsets of leukocytes. While the function of some of these cells is still elusive, like neutrophils which are essential components of the RCC microenvironment, others have well-defined roles in tumor progression. For example, tumor-associated macrophages (TAMs) are known for their immunosuppressive action, which is associated to the secretion of inhibitory cytokines, the generation of reactive oxygen species, regulatory T cells (Treg) development, and the induction of angiogenesis [6, 8, 9]. Likewise, myeloid-derived suppressor cells (MDSCs) have been reported preventing the formation and execution of an effective antitumor immune response by the inhibition of effector T-cell function and the induction of Tregs maturation [6, 7], besides the inhibition of DC maturation and DC-induced T-cell activation and antitumor cytotoxic T lymphocyte (CTL) [10].

The role of the immune system in RCC is not only observed at the cellular level but also through inflammatory mediators, that is, through the action of cytokines and chemokines which act in tumor and stroma cells [11–13]. These mediators in RCC and stroma cells lead to survival, proliferation, and migration and favor angiogenesis and metastasis [11–13]. Thereby, the modulation of immune system effectors has shown therapeutic potential. In fact, strategies involving immune checkpoint inhibitors, immunotherapy, and antagonists of chemokine receptors have proven clinical efficacy [14, 15].

Herein, we summarize relevant information about the role of natural and adaptive immunity in the development of RCC. Additionally, we describe cytokine and chemokine intracellular signaling pathways and mention how all knowledge has been useful for identification and advancement of therapeutic approaches for RCC.

## 2. Natural and adaptive immunity in RCC

The tumor microenvironment is a complex structure composed by several mediators that are involved in the cell signaling. The activation profile of extracellular matrix, fibroblasts, immune cells, blood vessels, and endothelial cells is essential to the pathogenesis of cancer, as well as to define therapeutic approaches [16–18]. Resident nontumor cells or infiltrated cells at tumor sites may even suppress the development of RCC. However, tumor cells can avoid the immune response coordinating changes in these cells and stimulating the secretion of immunosuppressive factors for tumorigenesis, pro-inflammatory cytokines including angiogenic factors, thus ensuring the supply of nutrients by newly formed, blood vessels, which further allow for tumor growth [19, 20].

In addition, the profile of soluble factors, such as cytokines and chemokines, present in the tumor microenvironment may undergo dual polarization allowing either tumor growth

(leading to progressive disease) or its suppression (leading to regressive disease). Immune cells could interact with tumor cells, especially through immunosuppression, via cell-cell contact, or by the release of factors that maintain a supportive environment for tumor growth. Through some soluble mediators, such as tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), immune cells can stimulate other cells, such as fibroblasts, to produce proteolytic enzymes for extracellular matrix remodeling and collagen, facilitating the spread of metastases. It may also stimulate the involvement of endothelial cells, which become able to form new vessels [18, 21, 22]. On the other hand, TNF $\alpha$ , which is mainly produced by macrophages, has also an important role in the recruitment of other immune cells to the tumor site where they will be able to assemble a response against the tumor. Thus, the constant interference of mediators may favor/inhibit an adequate environment for tumor maintenance and growth [18, 21].

Indeed, tissue repair promoted by inflammation is self-limited, and the imbalance on this process may be pathogenic, giving inflammatory cells either a beneficial and/or a detrimental role in the pathogenesis of various diseases, including chronic inflammation and neoplasia [23]. The TAMs have at least two well-described states of polarization, according to immunological competences. While M1 (classically activated) macrophages can inhibit tumor growth, M2 (alternatively activated) macrophages stimulate tumor growth [24–26]. The M1 polarization is functionally characterized by the release of pro-inflammatory cytokines (interleukin [IL]-1 $\beta$ , IL-6, and TNF $\alpha$ ) and reactive nitrogen/oxygen species (RNI/ROS) acting as microbicidal and tumoricidal [24]. In the tumor context, some authors suggest that macrophages in the initial phase of tumorigenesis can naturally inhibit tumor growth, eradicate tumor cells, and stimulate the immune response. In contrast, M2 macrophages may favor neoplasia by producing anti-inflammatory cytokines that suppress the cellular response, release vascular endothelial growth factors (VEGFs), responsible for angiogenesis, and release transforming growth factors (TGF- $\beta$ ) [27].

The different subtypes of T lymphocytes are also recruited into the tumor microenvironment and interact with tumor cells, which may induce tumor cell death, becoming anergic or even suppressing the immune response against the tumor [28, 29]. T CD8<sup>+</sup> lymphocytes, also known as cytotoxic T cells, play an important role in the adaptive immunity in response to tumor-specific epitopes. Together with NK and Natural killer T (NKT) cells, T CD8<sup>+</sup> cells induce tumor cell death by apoptosis through the secretion of cytotoxic factor, such as granzymes and perforins. Besides, when the TCD8 lymphocyte is activated, it secretes IFN- $\gamma$  and IL2, activating other TCD8 cells and M1 macrophages, skewing the inflammatory response toward a Th1 profile. However, RCC and some types of tumor-infiltrating immune cells, such as MDSCs, present in the tumor microenvironment can suppress antitumor response T cell mediated by the expression of programmed death ligand-1 (PD-L1, also known as B7-H1 or CD274) [30, 31].

CD4 T lymphocyte is endowed with great plasticity, being able to differentiate into Th1, Th2, Th3, Th17, and Treg subtypes. Treg cells play a critical role in the control of the acute phase of the inflammatory response through immunosuppression. High levels of Treg in the tumor microenvironment are considered as poor prognosis for tumors, for example, in renal and pancreatic tumor, due to the recruitment of other immunosuppressive cells and the stimulation of the angiogenic process [29, 32]. MDSCs are involved in chronic inflammation processes, which are recruited by tumor or Treg, increasing immunosuppression in the tumor microenvironment [33].

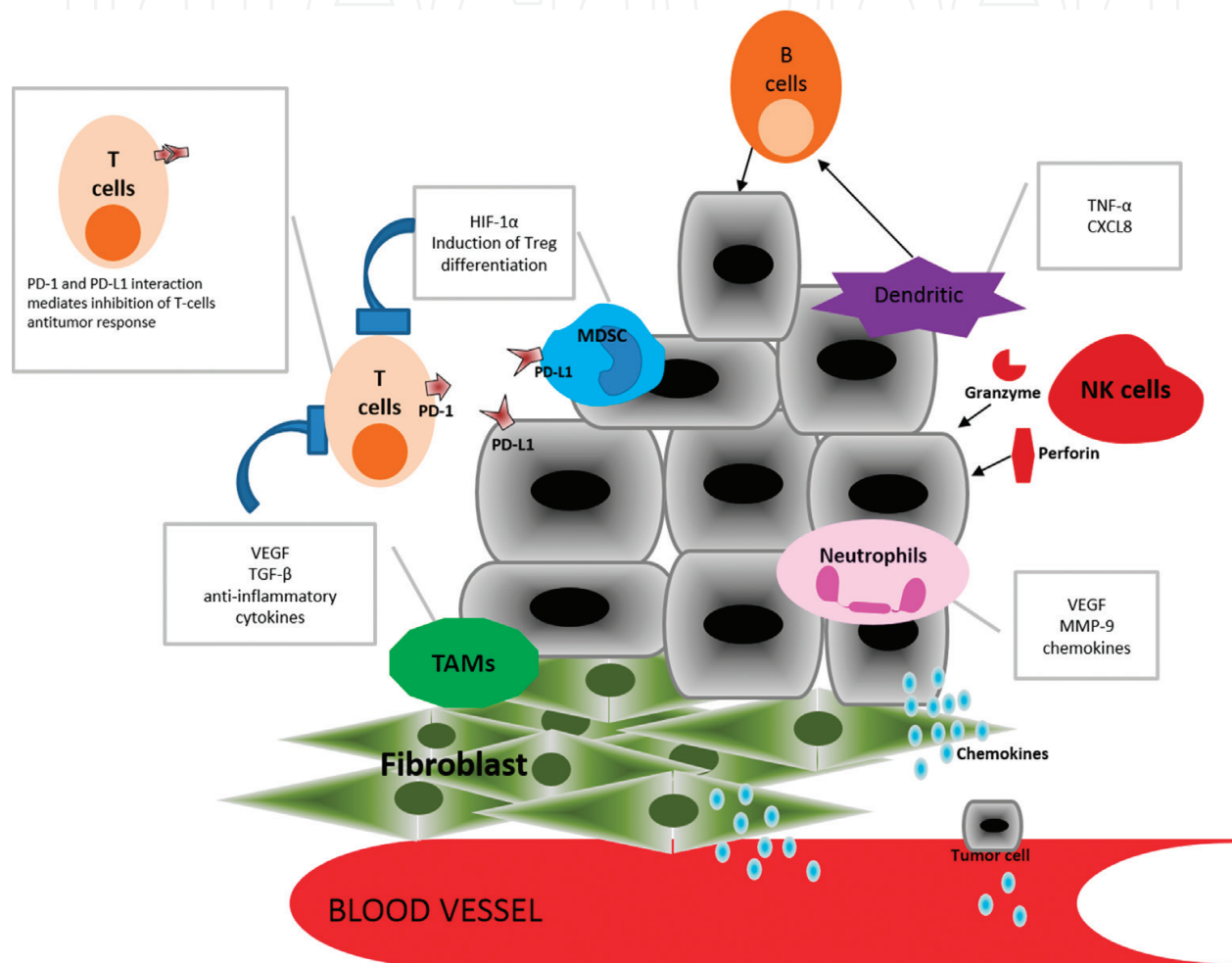
A common feature of almost all solid tumors is hypoxia that could lead to the stability of the HIF-1 $\alpha$  transcription factor (hypoxia-inducible factor-1 $\alpha$ ). HIF-1 $\alpha$  can bind to a hypoxia-responsive element in the PD-L1 promoter leading to PD-L1 expression, not only on tumor cells but also on MDSCs, macrophages, and DCs within the tumor microenvironment [34].

PD-L1 binds with PD-1 present on the cell surface of activated T cells leading to proliferation blockade, dysfunctional response, and T-cell death. CTLA-4 (CTL-associated antigen 4), a receptor also present in T cells, is the best studied inhibitory molecule, well known for its capacity of blocking T cell-mediated immune responses [35, 36]. CTLA-4 competes with CD28 for CD80 or CD86 binding. When the CTLA-4/CD80 or CD86 interaction occurs, the cell becomes anergic and dies. The overexpression of CTLA-4 is involved in several neoplastic, inflammatory, and autoimmune diseases [37]. The inhibition of T response allows for RCC to avoid effectors of immunologic control and facilitates invasion and metastasis. Accordingly, PD-L1 overexpression is related to worse prognosis for metastatic RCC, and it is therefore an important target for drug discovery [38, 39]. New drugs based on monoclonal antibodies, such as anti-PD-1 and anti-CTLA-4, have been strongly explored in clinical trial and proven to be efficacious to combinatorial treatments [39]. Indeed, immunotherapy is a promising therapeutic strategy for different types of cancers, but it is often not sufficient to control tumor growth [40]. Several therapeutic strategies based on innate, adaptive, humoral, or cytokine immune system responses have been studied in order to combat tumor cells in the host [41–45].

Studies using allogeneic DCs in metastatic Renal Cell Carcinoma (mRCC) and melanoma patients are widespread due to the ability of these cells to mediate the cell signaling between the innate and adaptive immune response. As professional antigen-presenting cells (APCs), these cells phagocytose tumor cell particles, process them, and further present their epitopes to further activate effector lymphocytes [46]. APCs play a crucial role in coordinating the immune response, where the imbalance between populations of macrophages, immature, and mature DCs significantly affects the immune response against solid tumors [47]. Both DCs and macrophages can be activated by some microbial stimulus or cytokines in an inflammatory environment. DCs and macrophages could differentiate from monocytes, according to the tissue environment, where cytokines such as IL4 and macrophage colony-stimulating factor granulocytes (GM-CSF) may induce differentiation, followed by TNF $\alpha$ -induced maturation [47]. In the tumor context, the inflammatory profile present at different stages of disease development may contribute to or eradicate the pathogenesis [47]. The International Society for the Biological Therapy of Cancer (iSBTc), together with the Society for Cancer Immunotherapy (SITC), has been discussing the theme with the purpose of advancing the critical understanding of the involvement of inflammation during pathogenesis and cancer treatment [48, 49]. In fact, the dynamics of tumor progression along with immunoediting has been studied for years, through the realization of which cells, molecules, and pathways of the immune system are engaged at different steps of the evolution of cancers. Nonetheless, an integrative picture of the whole process is still missing [50, 51]. Vesely et al. described several interactions between innate and adaptive immunity in cancer, suggesting a dynamic immuno-delivery model, where cells such as M2 macrophages, MDSC, Th17, Treg, and TCD8<sup>+</sup> overexpressing CTLA-4 receptor are present in chronic inflammation and favor the processes related to tumor progression [52]. In contrast, NK, NKT, TCD8<sup>+</sup>, TCD4<sup>+</sup>, M1 macrophages, DCs, T $\gamma$  $\delta$  cells and IL12 and IFN-gamma



cytokines are important for equilibrium and elimination phases of tumor control [52]. Several cytokines are involved in the differentiation process of immune cells. Interferon-gamma (INF-gamma) belongs to interferon family and is a natural glycoprotein that shows antiviral, antiproliferative, and immunomodulatory properties. INF-gamma plays a key role in the tumor microenvironment, where it aids in tumor eradication. This cytokine is able to recruit and induce the proliferation of T lymphocytes in the tumor microenvironment, to activate innate immunity cells, rendering them cytotoxic, besides polarizing the Th1 response from T CD4



**Figure 1.** Dual role of natural and adaptive immunity in RCC. Innate immune cells (dendritic cells, macrophages, NK cells, neutrophils, and MDSCs) and adaptive immunity (B and T cells) in the tumor microenvironment may undergo dual polarization allowing either tumor growth (leading to progressive disease) or its suppression (leading to regressive disease). Dendritic cells are primed by tumor antigens, which are then presented to T and B cells for adaptive responses. On the other hand, dendritic cells can directly drive tumor angiogenesis through the release of pro-angiogenic cytokines such as TNF $\alpha$  and CXCL8. Similarly, neutrophils in the tumor microenvironment are able to release pro-angiogenic factors and chemokines that could contribute for cancer progression and metastasis. T CD8 $^{+}$  lymphocytes, also known as cytotoxic T cells, play an important role against tumor cells under response to tumor-specific epitopes. However, the PD-L1/PD-1 interaction on CD8 $^{+}$  T-cell surface induces cellular energy suppressing the effector response, leading CD8 T cell to death. Failure of CD8 $^{+}$  T cells to kill tumor cells involves signals from multiple cells including MDSC, Treg, and TAMs. NK cells are characterized by a high cytolytic capacity against transformed cancer cells by the secretion of granzyme and perforin. Tumor cells and fibroblasts also produce survival/growth-promoting chemokines. Metastatic cancer cells are facilitated by the upregulation of particular chemokine receptors (such as CXCR4) by tumor cells, which enables them to migrate to secondary tissues where the ligands are expressed.

lymphocytes [53, 54]. IL1-beta is one of the major cytokines involved in the pro-inflammatory response, which is synthesized by several immune cells, such as monocytes, macrophages, DCs, B lymphocytes, NK cells, among others. It has similar activities as described for TNF $\alpha$ , favoring tumor invasion and the angiogenic process, as well as favoring vascular permeability and facilitating the recruitment of immune system cells to the tumor microenvironment [55]. However, TNF $\alpha$  is the main mediator of the acute inflammatory response, being secreted primarily by macrophages and T cells. TNF $\alpha$  causes vascular endothelial cells to increase the expression of leukocyte integrins inducing chemotaxis. In addition, TNF $\alpha$  also acts on phagocytic cells, which characterizes an autocrine effect, since macrophages, apart from secreting TNF $\alpha$ , may respond to the stimulus itself, releasing IL1-beta [56, 57]. IL12 is secreted primarily by macrophages, DCs, monocytes, and neutrophils. It has action in the activation of cytotoxic NK cells and TCD8 lymphocytes, but its main function in the antitumor activity is involved in the activation and proliferation of T lymphocytes and NK cells, which induces the production of IFN-gamma. Moreover, IL12 and INF-gamma together are able to differentiate T-helper cells into Th1 cells [58, 59]. IL6 is synthesized by mononuclear phagocytes, such as macrophages and also by some activated T cells and by other cell types that are not part of the immune system in response to microorganisms or IL1-beta and TNF $\alpha$  stimuli [60]. IL10 is a cytokine known to be anti-inflammatory, synthesized in the form of monomers of 18–20 kDa, being functional in the form of homodimers. This cytokine can be produced by Th2 lymphocytes, monocytes, and epithelial cells. Its main action is to suppress the synthesis of several inflammatory cytokines such as IL1-beta, TNF $\alpha$ , IL-6, IL-8, and IL-12, as well as hematopoietic growth factors (GM-CSF, G-CFS) and macrophage colony-stimulating factor (M-CFS). In addition, IL-10 can inhibit the synthesis of nitric oxide, gelatinase, and collagenase, avoiding tissue injury [61]. Although its role in the tumor context remains unclear, IL-17 is a pro-inflammatory cytokine secreted by Th17 lymphocytes, which regulates NF $\kappa$ B and MAPK activities. It is constantly involved in the acute phase of inflammatory diseases, such as autoimmune diseases, and it is associated with poor prognosis in patients with RCC [62, 63].

Taken all these studies together, the dual role of natural and adaptive immunity in RCC is evident (**Figure 1**).

### 3. Cytokines and chemokine intracellular signaling pathways

Physiologically, inflammation is a mechanism of tissue reaction for elimination, neutralization, and destruction of the cause of aggression, as injurious stimuli such as microbial pathogens, irritants, or toxic cellular components [64]. Cells of the immune system including monocytes, macrophages, neutrophils, basophils, DCs, mast cells, T cells, and B cells play a role in this process [64]. These events are in turn controlled by a host of extracellular molecular regulators, including members of the cytokine and chemokine families that mediate both immune cell recruitment and complex intracellular signaling control mechanisms; as a result, cells assemble and disassemble a complex array of signaling pathways as they move from inactive to dedicated roles within the inflammatory response site. Disruption of these pathways triggers inflammatory disorders that could contribute for the development of some diseases as kidney cancer and other types of cancers [65].

Key pro-inflammatory cytokines in kidney cancer include interleukin-1 beta (IL-1 $\beta$ ), IL-6, and TNF $\alpha$ , all of which signal via the type I cytokine receptors that are structurally divergent from other cytokine receptor types [66, 67]. IL-1 signaling starts through its binding to its receptor composed of two subunits, interleukin 1 receptor type I (IL-1RI) and interleukin 1 receptor accessory protein (IL1RAP) [68, 69]. Signaling proceeds with TIR adaptor and MyD88 by recruitment of IL-1R-associated kinases (IRAKs), which promote TNFR-associated factor 6 (TRAF6) polyubiquitination via lysine 63 linkages. Subsequently, TRAF6 interacts with the TAK1/TAB1/TAB2 complex that allows NF $\kappa$ B nuclear translocation (p65/p50) resulting in pro-inflammatory gene expression [69]. Also, TAK1/TAB1/TAB2 complex triggers the activation of the mitogen-activated protein kinases (MAPKs), c-JunN-terminal kinase (JNK), and p38, which induce the expression of pro-inflammatory genes. Similarly, TNF $\alpha$  binding to TNFR1 results in NF $\kappa$ B nuclear translocation, MAPKs, JNK, and p38, but signaling is coordinated by complex I (TRADD/TRAF2/RIP) [67, 70]. Importantly, TNF $\alpha$  signaling following receptor internalization is thought to be pro-apoptotic, via the formation of complex II (TRADD/FADD/Pro-Caspase-8) [67]. On the other hand, IL-6 binds to the FIII domains of the IL-6R chains, unleashing its signal via the gp130 proteins [71, 72]. Consequently, Janus kinases (JAKs) are recruited to the receptor, phosphorylating it and themselves, triggering STAT3 activation and transcription of pro-inflammatory genes and intracellular adhesion molecules [64].

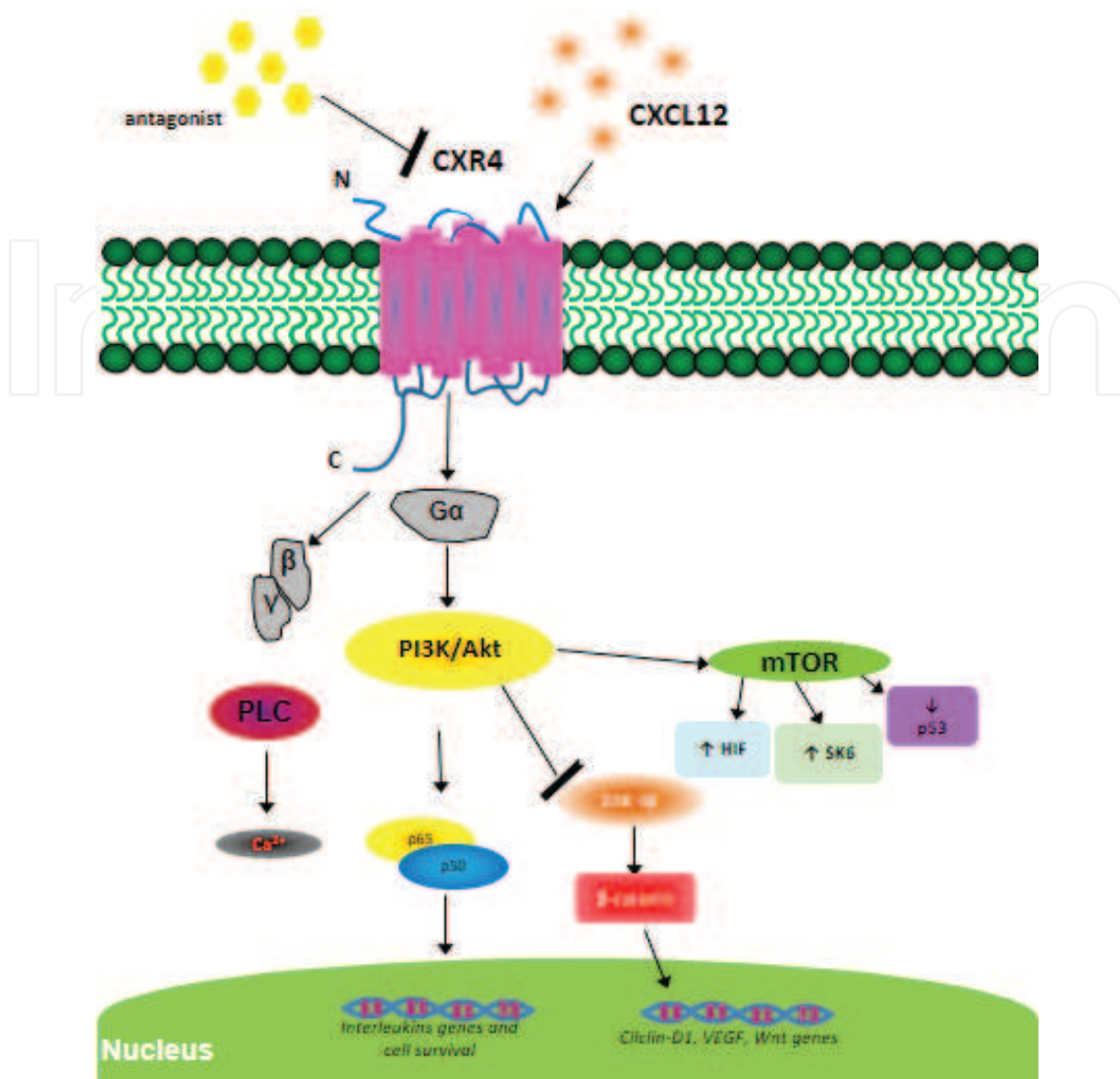
Currently, it is not fully understood how the signaling triggered by these cytokines contributes to tumor progression, but high serum levels of these pro-inflammatory cytokines are associated with advanced disease [73]. Some evidence has arisen, as follows. It is well known that angiogenesis is stimulated by inflammatory mediators in the tumor microenvironment, such as those expressed by TAMs [74]. Interestingly, TAMs isolated from RCC tumors express high levels of IL-1 $\beta$ , TNF $\alpha$ , and IL-6 [75]. In addition, mouse models have demonstrated that the inhibition of IL-1 $\beta$  signaling reduced tumor blood vessel formation [76] and IL-1 $\beta$  mediates metalloproteinase-dependent RCC tumor cell invasion through the activation of cytosine-cytosine-adenosine-adenosine-thymidine (CCAAT) enhancer binding protein b [67]. Regarding TNF $\alpha$  signaling, many studies associated it to chemokine overexpression in tumor and nontumor cells [77, 78]. Moreover, TNF $\alpha$  plays an important role in the progression of RCC by inducing epithelial to mesenchymal transition and CD44 expression, which may be involved in the resistance to the sunitinib treatment [66]. There is no direct correlation between IL-6R and RCC development; however, RCC cells express high levels of IL-6, and its signaling activity seems necessary for carcinogenesis, tumor progression, and tumor evasion of the immune system. STAT3 activation by IL-6 promotes tumorigenesis by preventing apoptosis while enhancing proliferation, angiogenesis, invasiveness, and immune evasion [79]. For example, activated STAT3 induces HIF-1 $\alpha$ -mediated VEGF expression in human RCC cell [79].

Besides these pro-inflammatory cytokines, other mediators act as crucial players in RCC. Chemotactic cytokines or chemokines are responsible for the recruitment of cells from both the innate and adaptive immune systems to the site of injury or infection [64]. Chemokines induce integrin expression, such as the  $\beta$ 2-integrin lymphocyte function-associated antigen (LFA-1), in target leukocytes, thus acting in the arrest of these cells and favoring diapedesis through the endothelium [71]. Despite this, primary chemotaxis action, chemokines, and their receptors are physiological relevant in many biological process, such as the initiation of adaptive immune responses, immune surveillance and the migration, proliferation, and



survival signals in multiple cell types [64]. Chemokine signals are transduced through binding to members of the seven-transmembrane, G-protein-coupled receptor (GPCR) superfamily [80]. GPCRs exist as a heterotrimer containing three subunits:  $\alpha$ ,  $\beta$ , and  $\gamma$ . In its inactive form, the G protein is complexed in  $\alpha$ ,  $\beta$ , and  $\gamma$ , with guanosine diphosphate (GDP) fixed to the  $\alpha$  subunit. Once stimulated by a receptor activated by its ligand, the  $\alpha$  subunit exchanges its GDP for Guanosine-5'-triphosphate (GTP) [81]. This causes the dissociation of  $\alpha$  which separate  $\beta$  and  $\gamma$  subunits by interacting with an effector protein or ion channel in order to stimulate or inhibit secondary intracellular messengers [81]. CXCR4 is well known for its role in the homing of progenitor cells into the bone marrow and, recently, associated with poor RCC prognosis, and it is mainly coupled to the  $G_{ai}$  subunit, which, after dissociation of the  $G_{\alpha\beta\gamma}$  complex upon CXCR4 stimulation (**Figure 2**), is traditionally been regarded as the major signaling subunit, inhibits adenylyl cyclase activity, and triggers MAPK and phosphatidylinositol-3-kinase (PI3K) pathway activation [82]. The  $G_{\beta\gamma}$  subunits, in turn, lead to the activation of phospholipase C (PLC), causing the hydrolysis of the phospholipid membrane phosphatidylinositol 4,5-bisphosphate (PIP2) in inositol 1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG) [13, 82]. IP3 can bind to channels of the endoplasmic reticulum, inducing the mobilization of  $Ca^{2+}$  ions [83, 84]. This could also be considered a downstream effect of  $G_{ai}$  activity, since the inhibition of  $G_{ai}$  activity by its potent inhibitor pertussis toxin has been reported to lead to a decreased  $Ca^{2+}$  mobilization from intracellular stores [85]. CXCR4 can also act by interaction with other  $G_{\alpha}$  subunits, that is,  $G_{aq}$  or  $G_{\alpha 12}$ , each of which has been associated with different intracellular signaling cascades [86]. Indeed, chemokine receptors also activate signaling pathways independent of G proteins, including p38MAPK and JAK/Stat to regulate cellular processes such as migration and gene transcription [87, 88].

Regarding the signals triggered by pro-inflammatory mediators in RCC, CXCL12 chemokine and its receptors CXCR4 and CXCR7 have gained prominence, since this pathway, which is associated with chronic inflammation, is upregulated in RCC [65, 89, 90]. In RCC cells and in other tumor cells, these chemokines activate the PI3K/Akt pathway; consequently, many downstream elements of the Akt pathway are regulated, leading to tumor cell survival [13, 91, 92]. Frequently, NF $\kappa$ B nuclear translocation is observed following transcription of various apoptosis inhibitors and cell-cycle-promoting genes [93], but can be activated through other pathways, such as PKC [94]. Other downstream targets of Akt include procaspase-9 and the pro-apoptotic Bcl-2 family member, BAD (Bcl-2/Bcl-XL-antagonist, causing cell death), both of which are inhibited upon phosphorylation. Other consequence of Akt activation is the inhibition of members of the FKHR (forkhead in rhabdomyosarcoma) family of transcription factors, which induce the transcription of numerous apoptotic genes [95, 96]. Besides, Akt signaling could induce p53 degradation and inhibition of GSK-3 $\beta$  (glycogen synthase kinase-3 $\beta$ ), leading to stabilization of  $\beta$ -catenin, resulting in the downstream inhibition of negative regulators of cell cycle and the activation of cell-cycle-promoting genes [97]. mTOR (mammalian target of rapamycin) activation is also induced by Akt, which leads to p70S6K (p70 S6 kinase) activation and thus enhances protein translation of numerous cell-growth regulators [13]. Furthermore, intracellular events triggered by the activation of chemokines receptors lead to ERK1/2 signaling, following the inhibition of procaspase-9 and BAD [98, 99], the induction of transcription factors involved in cell-cycle regulation, and differentiation, thereby promoting cell proliferation [100]. Other MAPKs, including JNK, have also been implicated in chemokine-induced proliferation signaling [101]. Also, HIF-1 $\alpha$  may be



**Figure 2.** Signaling induced by CXCR4 via G protein. CXCL12 binding causes the dissociation of  $\alpha$  which separate  $\beta$  and  $\gamma$  subunits; as a result,  $\beta\gamma$  PLC is activated leading to calcium mobilization. PI3K/Akt activation by  $G\alpha$  triggers a transduction signal that contributes with cell survival, proliferation, and migration, which is associated with RCC disease. In contrast, the antagonist of CXCR4 blocks CXCR4 intracellular pathway.

induced by chemokines signaling that contributes to VEGF expression, which is known to be the inducer of CXCR4 expression [101]. It is important to mention that not all of these pathways have been studied in detail in RCC cells, but there is strong evidence of their involvement in the development of this disease. For example, Rac1 was not previously reported to be involved in RCC development, but some studies have shown its role in controlling tumor cell growth and chronic kidney disease [102]. In summary, CXCL12, CXCR4, and CXCR7 release result in the activation of transcription factors involved in antiapoptotic mechanisms, cell-cycle regulation, and growth factor production, favoring tumor growth and metastasis.

Also, there are some particularities regarding CXCR7 role in RCC, which are independent of GPCRs. CXCR7 plays a role as a decoy for CXCL12, promoting some CXCL12 $\alpha$  accumulation and triggering a differential signaling by CXCR4 [103, 104]. Besides, CXCR7 interacts and signals by  $\beta$ -arrestin in a ligand-dependent manner [83, 104].

Obviously, here we emphasize intracellular events in tumor cells. However, while some processes take place within the tumor cells, others would occur in the stroma: it is the synergism between these responses that contributes to the progression of the disease. RCC and other tumors interact with the surrounding tumor stroma through a variety of cytokines, chemokines, and growth factors [84]. The tumor chemotactic environment recruits inflammatory cells including neutrophils, macrophages, and lymphocytes. Although initially these cells may have a protective antitumoral role, as displayed by neutrophils, which have a higher cytotoxic activity against poorly metastatic cells, secondarily neutrophils could contribute to cancer progression. Leukocytes can produce cytokines, growth factors, and MMPs that enhance growth, proliferation, and angiogenesis, as exemplified by the TAMs, which release growth and angiogenic factors (e.g., VEGF) and basic fibroblast growth factor [105]. Thus, cellular communication by paracrine and autocrine chemokine/cytokine signaling contributes for the survival and growth of metastatic cells. In other words, stroma cells may support tumors at the same time as that tumor cells in turn modulate the microenvironment within which they reside.

#### 4. Immunomodulatory molecules as new therapeutic targets for RCC

Among the standard drugs for metastatic RCC treatment, the most effective regimens include the combination of targeted therapy agents and antiangiogenic agents (tyrosine kinase inhibitors, such as sorafenib, sunitinib, pazopanib, and axitinib), or an antiangiogenic antibody routinely employed in combination with interferon alpha (bevacizumab) [106, 107], and antiproliferative agents (mTOR inhibitors, such as temsirolimus and everolimus) [106, 107]. Despite the effectiveness of these therapies, resistance to antiangiogenic therapy almost always occurs and are associated with high toxicity and, consequently, serious adverse events.

Considering all knowledge previously mentioned in this chapter, it is not surprising that agents altering the immune response in order to prevent tumor growth and metastases have gained space in this scenario [12, 14, 108]. Known as immunomodulators, these molecules have been studied for some time now [109]. Among the first representatives of this class are IL-2 and interferon-alpha (IFN- $\alpha$ ). IL-2 is a naturally occurring cytokine, which have antitumor activity by the induction of proliferation of NK cells, lymphokine-activated killer cells (LAKs), and other cytotoxic cells [110, 111]. IFN- $\alpha$  is also a molecule that had its clinical effect for the treatment of RCC demonstrated years ago [109, 112]. It is a pleiotropic cytokine with immunomodulatory activities that is able to induce the differentiation of monocytes into highly activated DCs, which are particularly effective in recognizing complex antigens and inducing T- and B-cell immunity and thus participate in the generation of antitumor T-cell immunity [112]. However, cytokine immunotherapies work effectively only in a minority of RCC patients and currently are not considered for the standard treatment of RCC due to their high toxicity.

In this context, cytokines have been exploited. Combination therapy using an antihuman IL-6R antibody with interferon has been suggested as a novel therapeutic approach for the treatment of RCC. Besides, an IL-6R-neutralizing antibody, tocilizumab, in combination with sorafenib suppressed tumor growth and inhibits angiogenesis *in vivo* more efficiently than sorafenib alone [113, 114]. Similarly, infliximab, an anti-TNF $\alpha$  monoclonal antibody that prevents TNF $\alpha$  binding to its receptors, TNFR1 (p55 receptor) and TNFR2 (p75 receptor) and causes cell death via complement-mediated lysis through interaction with membrane-bound

TNF, has been considered for RCC treatment [115]. However, treatments with anti-TNF $\alpha$  monoclonal antibody showed varying results in independent studies, probably due to reactions given by this cytokine to different conditions, environmental and genetic factors and/or other unknown or unexplained factors [116]. Besides anti-TNF $\alpha$  strategies, targeting IL-1 $\beta$  has also been reported as possible therapy for RCC [117].

Promising candidates for RCC treatment have also been designed to specifically target chemokines and their receptors. One of the most widely studied compounds is AMD3100 which is thought to specifically block CXCR4 signaling [118] and that acts directly in RCC tumor [91] cells as well as local antitumor immune response, by impairing Tregs function [119], know to suppress a whole range of immune cells including B cells, NK cells, NKT cells, CD4+ or CD8+ T cells, monocytes, and DCs [119]. Also, many other chemokine antagonists have also shown potential for clinical application in cancer treatment and could be useful for RCC treatment in the future. For example, anti-CXCR7-12G8 and CCX77, CXCR7 inhibitors or CTCE-9908, which is a peptide analog of CXCL12 and an active inhibitor of the ligand, has shown promising results as well as tolerated drug that stabilized disease in early clinical trials for late-stage cancer patients [12, 120].

Recently, antibodies that inhibit T-cell coinhibitory receptors have emerged as therapeutic promises not only in the treatment of RCC but also in other tumors by inhibiting T-cell regulatory activity and increasing the antitumor immune response [14, 121]. Nivolumab and pembrolizumab (anti-PD-1) [14, 122], avelumab, and durvalumab (anti-PD-L1) are in late-stage clinical development for a number of indications [14, 123], besides the first in its class, ipilimumab (anti-CTLA-4), already approved for use in a number of indications [14, 124].

Finally, the close relationship between cancer and immune system has suggested that current drug therapies used to treat inflammatory diseases or particular types of cancers could function as inhibitors of chemokine signaling and could therefore be redirected toward the treatment of other cancers [12, 116]. This hypothesis needs to be tested by further preclinical and clinical investigation, which elucidates how these drugs would act at molecular and systemic levels.

## 5. Role of immune system in kidney cancer and the future

The role of immune system in kidney cancer is becoming more clear, whereas new findings that arise from clinical trials and identification of additional predictive biomarkers increase our understanding of the tumor microenvironment. Looking to the future based on the knowledge we have today, the perspective is a better understanding of immune system in tumor stroma as well as in various steps in cancer growth and metastasis.

Regarding RCC therapy, a promising option is the combination therapy based on targeted agents (inhibition of mTOR or VEGF pathways associated with immunotherapies) or immunotherapy + immunotherapy, which would overcome tumor resistance, as well as to restore functional immune system cancer surveillance and response. Currently, there are many clinical trials investigating combination therapy: nivolumab (anti-PD-1) + ipilimumab (anti-CTLA-4) [125], pembrolizumab (anti-PD-1) + ipilimumab (anti-CTLA-4) [126], pidilizumab (anti-PD-1) + vaccine (DC/RCC fusion cell vaccine) [127], atezolizumab (anti-PD-L1) + bevacizumab (anti-VEGF) [127], nivolumab (anti-PD-1) + bevacizumab (anti-VEGF) [128], pembrolizumab (anti-PD-1) + pazopanib (TKI) [129], nivolumab (anti-PD-1) + sunitinib [130].



Also, cell-based therapies have become interesting, which includes adoptive T-cell therapies such as tumor-infiltrating lymphocytes (TILs), T-cell receptor (TCR), and chimeric antigen T-cell (CAR-T) therapy [127].

Other promising alternative of therapy for RCC is vaccine-based immunotherapy. AGS-003 is an autologous DC vaccine that is generated from host DCs in response to tumor mRNA [131], which is also to investigate in combination with sunitinib [132].

All these therapeutic investigations highlighted the importance of immune system in the future study about RCC. Information about immune system may be decisive for clinical decisions.

## 6. Conclusion

Immune system plays a role in kidney cancer, and this should be considered for both the understanding of the disease and the development of novel therapies.

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## Conflict of interest

The authors declare they have no conflicts of interest.

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