Chapter 12

The Inflammatory Microenvironment in Wilms Tumors

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Abstract

For the past several decades, the role of inflammation in different types of tumors has been well defined. The significance of inflammation including the presence of various immune cells and inflammatory marker analysis of tumors helped the clinicians to use new treatment methods, which lead to high cure rates but failed to do so in some tumors due to lack of information about the tumor microenvironment. Although the importance of inflammation in various adult malignancies has been well defined, by contrast, Wilms tumor (WT), the most common childhood kidney cancer, which represents 6% of all pediatric tumors, has

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not been well studied. Nearly 75% of the WT cases have been noticed in children less than 5 years of age with a higher incidence at 2 to 3 years. Thus, very little is known about the inflammatory microenvironment in the development of WT. This inflammatory microenvironment may initiate oncogenic transformation, and in some instances, genetic and epigenetic modifications in tumor cells can also generate an inflammatory microenvironment that further supports tumor progression. Thus, the tumor microenvironment is highly dynamic, and linking the modulating factors and various inflammatory cells with tumor progression is of considerable interest. Although to some extent the currently used WT treatment methods such as surgical removal, chemotherapy, and radiation therapy are successful, the youngest children are at high risk for the irreversible adverse side effects. Thus, there is a need for alternative therapy/therapies exposing the child to the minimum possible adverse effects. This chapter gives a special focus on the inflammatory microenvironment of human WT with a comprehensive picture of various immune cells and other inflammatory markers. This may aid in the use of new therapeutic targets for the efficacious treatment of WT with the combination of currently adapted therapies or alone.

Key words: Cancer; Immune cells; Inflammation; Microenvironment; Wilms tumor

Introduction

Following the transformation of a normal cell to malignant or tumor cell, the inflammatory mediators promote the tumor growth, by inducing the proliferation and the evading immune surveillance. The unregulated inflammatory microenvironment plays a central role in the initiation and progression of tumor. In general, inflammation is initiated by the recruitment of a wide range of immune cells that affect malignant cells through the production of cytokines, chemokines, growth factors, prostaglandins, reactive oxygen and nitrogen species, proteases, and other bioactive molecules, which can act in an autocrine and/or paracrine manner (1). Altogether, this environment with various factors is known inflammatory tumor microenvironment. These inflammatory markers are very critical components to establish a link between inflammation and cancer although the activation of these inflammatory markers is influenced by various factors. This inflammatory microenvironment progresses the tumor cells with endowed immunosuppressive properties. Hence, the immune destruction property has now been proposed as one of the "hallmarks of cancer." Thus, the role of inflammation and inflammatory microenvironment in cancer is generally accepted and is an essential component of many tumors even though its relationship with inflammation has not been demonstrated (1–4). So far, the molecular mechanisms involved in establishing this inflammatory tumor microenvironment were not clearly understood and established. This may be due to multifaceted role of inflammatory markers/mediators, such as cytokines, chemokines, oncogenes, enzymes, transcription factors, and immune cells, in the tumor

microenvironment. Till date, studies are still going on to elucidate the complete link between the cancer and the inflammation. For the past one decade, studies using knockout animals have unraveled to some extent the molecular mechanisms that link inflammation and cancer in adult-onset cancers but not in pediatric cancers (5). These studies show that the inflammatory microenvironment is very important in tumor development. The inflammatory conditions may initiate or promote oncogenic transformation, or genetic and epigenetic changes in malignant cells can also generate an inflammatory microenvironment that further supports tumor progression (2). It is important to note that the acute inflammation regresses the tumor growth, whereas the chronic inflammation progresses the tumor. Thus, there is a need to be a balance between antitumor immunity and tumor-promoting immune activity within a tumor microenvironment that consists of tumor cells, stroma (including fibroblasts and endothelial cells), innate immune cells, and adaptive immune cells.

What is Wilms tumor and what are the various components of Wilms tumor?

Wilms tumor (WT) is the most common pediatric kidney cancer, which represents 6% of all pediatric tumors, and 9 out of 10 kidney cancers in children are WTs. Nearly 75% of the WT cases have been noticed in children less than 5 years of age with a higher incidence at 2 to 3 years. It is the most common cause of a renal mass in a child and more prevalent in the people of African descent (6, 7). WT is an undifferentiated mesodermal tumor, which consists of variable amount of embryonic renal elements, such as blastema, epithelium, and stroma (8, 9). The etiology of this childhood tumor is largely due to genetic alterations or mutations in the *WT1*, *CTNNB1*, and/or *WTX1* genes.

Most of the WTs are unilateral and most often involve only one tumor, but it has been observed that around 5% to 10% of children with WTs have more than one tumor in the same kidney. Only about 5% of children with WTs have bilateral disease. Most often, the size of the WT is much larger than that of the kidney before they were diagnosed and metastasized to other organs (10).

The mechanism/mechanisms of this pediatric cancer development at present is less clear, and the whole etiology of these diseases is also not completely understood. In general, pediatric cancers will not arise from epithelial tissues and will have different causative mechanisms than adult tumors. It is assumed that most of the childhood cancers arise as a result of inherited and/or acquired genetic events during embryogenesis (11).

Although in general the currently used WT treatment methods such as surgical removal, chemotherapy, and radiation therapy are successful, young children are still at high risk for the irreversible adverse side effects. In addition, a considerable number of patients relapse (20–25%), and part of these tumors resist to current therapies and progress (12, 13). Thus, the main

challenge is better stratification and development of novel therapeutic targets/approaches to eliminate or minimize these side effects and deficiencies. Such novel approaches critically depend on the in-depth understanding of the tumor microenvironment and on the mediating factors responsible for WT progression. Hence, this chapter focuses on the inflammatory microenvironment of human WT with a comprehensive picture of various immune cells and other inflammatory markers. This may aid in the advent of new therapeutic targets for the efficacious treatment of WT with the combination of currently adapted therapies or alone.

Types of Wilms tumors

Based on the histology, WTs are categorized into two major groups.

Unfavorable histology (anaplastic WTs)

In these tumors, the tumor cells vary widely, and the nuclei is very large and distorted. This is called anaplasia. The anaplastic tumors are very hard to cure. In preoperative chemotherapy, such as in the International Society of Paediatric Oncology (SIOP) settings, also cases with chemo-resistant blastemal subtype, are considered at high risk of relapse.

Favorable histology

These are nonanaplastic tumors. Interestingly, more than 9 out of 10 WTs have a favorable histology. This type of tumors can easily be cured (10).

What is known about kidney cancer and inflammation?

There are not many studies available to relate WT and inflammation with the complete analysis of WT inflammatory microenvironment. In a comparative analysis of adult tumors, Vakkila et al. (14) reported that human WTs were infiltrated with macrophages and to a very less extent with T lymphocytes. This study was incomplete because it was confined to one or two immune cell markers. The other two different groups independently observed cyclooxygenase-2 (COX-2) expression in human WT ubiquitously in all cases, independent of the type (15) and stage (16) of neoplasm. However, these studies were again restricted to only one inflammatory marker, COX-2. The coexpression of the hypoxia-inducible factor (HIF-1α and its one of the target genes, vascular endothelial growth factor (VEGF), was reported in human WTs. This finding suggested the possible role of hypoxic cascade driving the tumor angiogenesis, growth, and progression (17). In addition, very early studies on isolation and culturing of tumor-infiltrating leukocytes (TIL) with different doses of cytokines in human WT comparing with other pediatric tumors were also reported (18). But none of these studies was able to give a comprehensive view of tumor microenvironment in human WT. It is therefore critical and relevant to know the whole picture of tumor microenvironment, whereas its role in

Wilms tumorigenesis has not been widely explored. Because there was not much information available about the complete analysis of the inflammatory microenvironment, we recently reported a comprehensive overview of various inflammatory markers and immune cells (qualitative and quantitative) in human WTs by immunohistochemistry (19).

Molecular links between WT and inflammation

Although there are a plethora of publications to link inflammation and adult tumor development, only few studies are available to relate the molecular links between WT and inflammation. Some of the recent findings are summarized below.

Immune cell infiltration

Our qualitative and quantitative immunohistochemical examination of immune cells in WTs (19) revealed infiltration of both adaptive and innate immune cells in tumors, similar to that previously reported in five WT samples (14) in a comparative study with adult tumors. However, our examination of a larger panel of tumors revealed that the extent of infiltration varied among tumors and among different histologically distinct regions within the same tumor, and also there was a difference in the in the quantity and infiltration pattern of adaptive and innate immune cells. Interestingly, while adaptive immune cells (T cells and B cells) were mostly localized to tumor stroma, innate immune cells [e.g., tumor-associated macrophages (TAMs), tumor-infiltrating neutrophils (TINs), and mast cells (MCs)], were not only predominantly localized to tumor stroma but also present in all other regions of the tumor. This different spatial localization suggested that a similar spatial pattern of chemical mediators, including chemokines and cytokines and other inflammatory proteins, might exist, either as a cause or as an effect of the presence of immune cells, which have been demonstrated to be recruited by, and also, in some cases, produce such mediators. To assess this possibility, we analyzed the expression and the intratumor localization of COX-2, HIF-1α, p-Stat3, p-ERK1/2, and the angiogenic marker, VEGF, in human WTs.

The following section describes the role of adaptive immune cells.

T lymphocytes

Human WTs were highly infiltrated with CD3+ T cells when compared with control kidney tissues in our earlier study (19). These T lymphocytes were almost absent in control kidney sections. Strikingly, although tumor stroma has many of the T lymphocytes when compared with other regions such as epithelium and blastema, the peritumoral area adjacent to tumor islands also has a huge number of infiltrating T lymphocytes. The peritumoral infiltration of this mononuclear T lymphocytes was greater than intratumoral (in blastemal, epithelial, and stromal regions) area of the tumor. Thus, this mononuclear T-cell infiltration was detected intensely in peritumoral region of the tumor in most of the cases we analyzed.

B Lymphocytes

B lymphocytes (CD20+) were also scattered in intra- and peritumoral region of WT with complete absence in control kidney sections, suggesting that these mononuclear lymphocytes (both T and B lymphocytes) followed the same kind of inflammatory cell infiltration pattern (19). Again, the density of CD20+ B-cell infiltration tended to be higher in peritumoral area than in intratumoral stromal region of the WT. In some of the tumors, we found only very few or absent in the tumor stroma, with aggregated infiltration of B lymphocytes found in most of the tumor-adjacent regions.

The role of innate immune cells is described below.

Macrophages

Although the intratumoral regions have infiltrating CD68+ macrophages (CD68MØ) in human WT, the majority of these CD68MØ were mostly dispersed extensively in tumor stroma in our earlier reported study (19). In contrary to the T and B lymphocytes, the CD68MØ within the tumor islands were also present in blastemal and epithelial regions although they were sparse when compared with tumor stroma. These CD68MØ were mostly in direct contact with the adjacent tumor cells in the invasive front. Surprisingly, although there were peritumoral CD68MØ, they were not comparable with the very highly infiltrated intratumoral regions. This observation is absolutely opposite to the lymphocyte (both T and B) infiltration, which we observed earlier. Very clear staining either in the membrane or in the cytoplasm was observed, with no staining in the nucleus. The spatial uniformity of the macrophage infiltration and density in the intratumoral region was maintained. But some tumors showed considerably less CD68MØ infiltration in some areas.

Neutrophils

TINs were identified in the intratumoral region of human WT (19). These TINs were mostly concentrated in the blastemal or epithelial regions to a lesser extent in the tumor stroma. There is a huge remarkable difference in the density of these cells in these different regions of the tumor. Most of these TINs were either intraepithelial or intrablastemal or, to some extent, were in the stroma, which is adjacent to the differentiated epithelial tissue. Overall, these TINs followed the tumorocentric distribution, concentrating mostly in neoplastic area as a massive infiltrate and diminishing its number or density distant from the neoplasm in almost all the WT cases in the current study. This is also true with anaplastic histology tumors, but the size of the neutrophils was slightly bigger in these tumors. TINs were not detected in the normal kidney.

Mast cells

MCs have been identified in the tumor microenvironment of various human neoplasias; we first confirmed that the MCs also infiltrate human WT (19). The infiltrating MCs were

distributed mainly in the invasive area of most of the human WTs. MCs were found in very small groups around neoplastic cells in tumor stroma and also in the peritumor areas but were almost absent in other intratumoral areas such as blastema and epithelium.

Together with these, various immune cell infiltration clearly demonstrates that the tumor inflammatory microenvironment is also present in human WT.

Inflammatory mediators

The inflammatory mediators can induce genetic and epigenetic changes that result in aberrations in critical biochemical pathways responsible for maintaining the cellular homeostasis, which leads to progression of cancer (1, 3, 4, 20). These inflammatory mediators may be of many types, such as cytokines, chemokines, free radicals, prostaglandins, growth factors, and enzymes such as COX.

COX-2

Positive immunoreactivity for COX-2 protein was observed in the entire tumor sections stained with diffuse moderate-to-strong cytoplasmic expression in the blastemal and the epithelial components and with very intense staining in tumor stroma. The infiltrating immune cells and other cells such as fibroblasts in the stroma were immune reactive for COX-2 protein. However, some of the tumors with anaplastic histology showed strong nuclear localization COX-2. The staining pattern and the intensity varied from tumor to tumor. Normal kidney samples showed weak to moderate staining in the cytoplasm of tubular epithelial cells. However, very weak or no staining was observed in the renal interstitial cells or glomeruli. We also investigated the correlation of COX-2 expression with the other inflammatory markers such as HIF-1, Stat-3, and VEGF. In addition, two different groups independently observed COX-2 expression in human WT ubiquitously in all cases, independent of the type (15) and stage (16) of neoplasm. COX-2 expression has been reported in other kidney cancers (renal cell carcinoma) (21), but not in pediatric tumors. In addition, Lee et al. (22) reported that the inhibition of COX-2 by SC-236 disrupted the tumor vascular mural cell recruitment and survival signaling in an orthotopic xenograft model of human WT. And another group (22) reported that the use of the same COX-2 inhibitor reduced tumor metastasis and inflammatory signaling during the blockade of VEGF in orthotopic SKNEP1 model of pediatric cancer.

HIF-1α

Very prominent nuclear localization of the HIF-1α protein expression was noticed in most of the cases evaluated in blastema, stroma, and epithelium along with negative HIF-1α expression in matched control kidney slides as reported earlier (19). In addition, some tumor specimens showed cytoplasmic granular staining in the cell cytosol and membranous (only in blastema) expression in blastemal and stromal compartments. The immune cell infiltrate of

tumor stroma was immunoreactive for HIF-1α protein as observed for COX-2 expression. Thus, the stromal expression of HIF-1α resembles the COX-2 expression. HIF-1α overexpression was reported in a significant proportion of WTs (23). In their study, they found no significant association between the expression of HIF-1α and clinicopathological variables in WTs resected following chemotherapy. In addition, the coexpression of HIF-1α has been reported with the angiogenic marker VEGF (17).

VEGF

VEGF expression was observed in most of the specimens (19). Although majority of the tumors showed VEGF expression in the infiltrating immune cells, connective tissue, or fibroblasts in tumor stroma similar to COX-2 and HIF-1α expression, but the blastemal and epithelial cell components were also immunoreactive, to some extent, in some tumor specimens. In the normal kidney samples, VEGF expression was observed in the proximal and distal convoluted tubules. Rowe et al. (24) reported that the anti-VEGF antibody suppressed primary tumor growth and metastasis in experimental models of WT. And the combination of low-dose topotecan and anti-VEGF antibody therapy suppressed the tumor growth and metastasis in experimental WT mice more durably than either agent alone (25). The immunohistochemical expression of VEGF-C and VEGFR-2 in the stromal and epithelial components of WT was reported (26) and indicated a potent unfavorable risk factor and directed the use of antiangiogenic treatment strategies to control the tumor growth.

Phosphorylated-Stat3 (p-Stat3)

The p-Stat3 expression was predominantly confined to the nucleus with almost undetectable cytoplasmic staining in all WT cases evaluated (19). Immunoreactivity of p-STAT3 was not detected in the control kidney tissue. Majority of the tumors showed the expression of p-Stat3 in the infiltrating immune cells in the tumor stroma, as well as in blastemal region, and these were very little or absent in epithelial cells. In addition, p-STAT3-expressing cells were found in the peritumoral area adjacent to the tumor islands. Moreover, the positive cells in this peritumoral area were found to be with stronger expression of p-STAT3 in the nucleus. Significantly higher nuclear immunoreactivity for p-Stat3 was also found in tumors compared with normal kidney sections. Furthermore, the expression of p-STAT3 was positively correlated with the TAM, CD3+ T cells, B cells, and inflammatory markers such as COX-2, HIF-1α, and VEGF. Zhang et al. (27) reported that the p-STAT3 expression in WT may correlate with progression and predict unfavorable prognosis and a new therapeutic target for metastatic WTs.

Phosphorylated pERK1/2

The expression of pERK1/2 protein was detected with very diffuse in the cytoplasm and more prominent staining in the nucleus in most of the WT cases (19), but to a small

extent, we were also able to see the cytoplasmic expression in normal kidney. However, the expression of phospho-mitogen-activated protein kinases (MAPK-/ERK1/2)-positive nuclei was observed in both peritumoral and tumoral islands. In most of the tumor cases, the expression was localized in tumor stroma with some extent in blastemal cells. Epithelial cell component of the tumors was almost absent with either cytoplasmic or nuclear pERK1/2 expression. The stromal expression was similar to COX2, HIF-1α, and VEGF expressions. The correlation between the p-ERK expression and other immune cell markers was also assessed. Significantly higher expression of pERK1/2 was observed in tumors than in control kidneys. It has been observed that the Wt1 ablation and insulin growth factor-2 (IGF2) upregulation resulted in WTs with elevated ERK1/2 phosphorylation in mice (28).

Inducible nitric oxide synthase (iNOS)

Although there are not many studies available on the expression of iNOS in WTs, we observed the iNOS expression (19) in tumor stroma with intense nuclear or cytoplasmic staining in most of the cases and diffuse cytoplasmic staining in blastemal cells of the tumor. The inflammatory immune cells within the tumor stroma were highly immunoreactive for iNOS in some of the WT specimens. Surprisingly, immunoreactivity for iNOS was also detected in the peritumoral area of some of the tumor sections. However, none of the epithelial cells expressed iNOS. In addition, areas around the tumor with neovascularization showed positive staining for iNOS. However, no significant immunoreactivity for iNOS was detected in the control kidney sections.

Nitrotyrosine (NT)

In our observation (19), WTs showed NT expression in the cytoplasm of the inflammatory immune cells of tumor stroma, as well as with much diffused cytoplasmic staining in the blastemal and epithelial regions of the tumors. The NT expression was observed very rarely in the peritumoral region. The expression was mostly localized within the tumor.

Chemokines and cytokines

Chemokines play an important role in tumor development and metastasis. The expression or secretion of these chemokines in the tumor microenvironment of various cancers, including breast, ovarian, pancreatic, melanoma, lung cancers, etc, have been reported. The expression of chemokines and their receptors is altered in many malignancies, and it leads to aberrant chemokine receptor signaling. Although the chemokine expression has been reported in various cancers, there is not much information available in human WTs. However, the role of ELR-CXC chemokine family members CXCL2 and CXCL7 and their receptor CXCR2 was expressed at the earliest stages of metanephric development in the rat, and signaling through this receptor was required for the survival and maintenance of the undifferentiated metanephric mesenchyme (MM) (29).

Other markers expressed in WTs

CITED1

In general, CITED1 is expressed at high levels in the condensed metanephric mesenchyme (MM) surrounding ureteric bud (UB)tips, is downregulated temporally as these cells begin to differentiate into early epithelial structures, and is not expressed in differentiated elements of the adult kidney (30). WTs arise from the undifferentiated renal progenitor cells. CITED1, which is a transcriptional regulator, blocks the metanephric mesenchymal-to-epithelial transition and is expressed in the blastema of both the developing kidney and WTs. The overexpression of CITED1 in a human WT cell line significantly increased proliferation in vitro, and mutation of its functionally critical transactivation domain (DCR2) significantly reduced proliferation (31). CITED1 expression was observed in blastemal cell populations of both experimental rat nephroblastomas and human WTs, and that primary human WTs presenting with disseminated disease show the highest level of CITED1 expression (32). Rivera and Haber (33) reported the Cited1 expression in the undifferentiated MM cells of WTs, and its expression was primarily confined to the nucleus. These studies suggest CITED1 as a marker of primitive blastema in WTs, and its persistent expression and altered subcellular localization in the condensed MM might have a role in WT initiation and progression. And another possibility is that persistent expression of CITED1 in metanephric blastema may have adverse developmental role in the pathogenesis of WTs.

B7-H1

A membrane glycoprotein, B7-H1, has been reported to act as an important coregulator of antigen-specific T-cell-mediated immunity (34, 35). This is normally expressed by the macrophage lineage cells and is aberrantly expressed by multiple human malignancies (34–36). Interestingly, tumor B7-H1 has been observed to induce T-cell apoptosis or anergy, thereby downregulating the host antitumoral immunity (34). B7-H1 expression has been observed in WTs, and its expression correlated with tumor biology and is associated with an increased risk of recurrence in patients with favorable-histology tumors (36). Because B7-H1 is involved in T-cell apoptosis, its expression in WTs may be related to inflammation. Thus, B7-H1 expression may be used as a prognostic marker, which indicates the aggressive behavior for favorable-histology WT. In addition, B7-H1 may be used to distinguish patients with favorable-histology WT, who require aggressive treatment and unfavorable-histology tumors, and who are at very low risk for disease recurrence and death and to avoid unnecessary overtreatment (36).

CD44

CD44 is also a membrane glycoprotein like B7-H1 expressed in a variety of cells, including those of epithelial, mesenchymal, and hematopoietic origin (37). CD44 expression has been observed in three different isoforms, such as CD44s, CD44v5, and CD44v10. The expression

of CD44s has been observed in all three components of the WTs. Among the isoforms, overexpression of CD44v5 in blastemal cells of WT correlated with tumor stage, clinical progression, and tumor-related death (37). Therefore, CD44v5 expression may be used as a good prognostic marker in identifying WT patients with a high tendency for distant metastases. Several studies have indicated that the increased expression of the CD44 gene is associated with metastatic disease. Studies by various other groups also indicated that the expression of CD44 isoforms may be a good prognostic marker for various cancers (38–40).

Carbonic anhydrase IX (CAIX)

CAIX is a membrane glycoprotein, which plays an important role in the growth and survival of tumor cells under normoxic, as well as hypoxic, conditions (23, 41, 42). CAIX (mRNA and protein) expression has been reported to be upregulated in the untreated and treated WTs when compared with normal kidneys and WT precursor lesions (nephrogenic rests) (23). There was no correlation between CA expression and clinicopathological variables, including metastatic status in postchemotherapy-treated WTs. Cellular localization studies in untreated WTs suggest that CAIX and HIF-1α are regulated by hypoxic and nonhypoxic mechanisms.

Role of immune cell infiltration with COX-2 pathway components

The expression of the inflammatory markers such as COX-2, HIF-1, iNOS, p-ERK1/2, and VEGF was predominantly localized to tumor stroma similar to the expression of TAMs. The codistribution of major inflammatory marker COX-2 with TAM infiltration in the tumor stroma was observed in our study (19). This study suggests that the infiltration of inflammatory immune cells and the expression of inflammatory markers in the tumor stroma are related. This observation suggests a correlation between the infiltrating immune cells and the activated cytokines and chemokines. This TAM infiltration was further confirmed (F4/80 expression) in the mouse model of WT (19). TAM infiltration is known to be induced by COX-2 in the tumor microenvironment (43), especially in the tumor stroma, and TAMs can also induce the expression of COX-2 (44). We have reported earlier that the colocalization of COX-2 and TAMs in the tumor stroma (19) may activate each other in the tumor microenvironment. The mechanisms responsible for the abundant COX-2 expression in WTs are that the infiltrating immune cells themselves could be overexpressing COX-2, or tumor fibroblasts may be generating COX-2 in response to macrophage infiltration, or fetal mitogen IGF2 may induce COX2 by MEK/ERK pathway (45).

Cross talk between immune cells and other markers in WT microenvironment

As indicated earlier, TAMs are also involved in the production of proangiogenic factors, such as transforming growth factor β and VEGF (46, 47), and of immunosuppressive chemokines and cytokines, such as interleukin 10 and prostaglandin E2, which contribute to

tumor angiogenesis (46, 48, 49). Thus, the TAM infiltration might play a significant role in the increased VEGF expression and also in the vascularization of the tumors. The correlation and localization of TAMs in the tumor stroma with the expression of various inflammatory protein markers, such as COX-2, HIF-1, p-ERK1/2, iNOS, and NT, suggest a functional association of TAM infiltration with the overexpression of these markers and vice versa in WTs (19) and demonstrate the existence of a highly inflammatory microenvironment in this disease.

Possible mechanism/mechanisms responsible for COX-2 pathway activation

Reports from our laboratory indicated that p-ERK1/2 was induced in mice, which are engineered to overexpress IGF2 and along with ablation of Wt1 gene, and also in human WTs (28), suggesting a role for the ERK signaling in WT development. The robust expression of COX-2 and p-ERK1/2 in tumors may be a consequence of IGF2 overexpression in WTs because IGF2-mediated COX2 expression has been reported in other tumors (45). Thus, the upregulated COX-2 expression creates an inflammatory microenvironment in WTs, which may be mediated by the enhanced p-ERK signaling is depicted in Figure 1. In the tumor microenvironment, COX-2 can also activate the expression of HIF-1 through its enzymatic product prostaglandin E2 (45, 50). Furthermore, this upregulated expression of p-ERK1/2 stabilizes the HIF-1α protein by preventing its degradation via the blockage of prolyl hydroxylase activity, which regulates HIF-1 or activates HIF-1α protein (Figure 1). Spatially similar expression of COX-2 and HIF-1 was observed in WTs (19), suggesting the role of COX-2 in HIF-1 activation. COX-2 activation of HIF-1 can also occur through hypoxia (17, 51) or hypoxia-independent mechanisms (52), with the involvement of p-ERK1/2 (53). In addition, it has been reported that PGE2, the end product of COX-2 pathway, can also enhance HIF-1 transcriptional activity (51). HIF-1 can also directly upregulate the expression of COX-2 during hypoxia (54) and thus form a feedback loop to continually activate the COX-2 pathway (Figure 1). Hence, it may be assumed that IGF2 affects the inflammation, hyperproliferation, and angiogenesis in WTs by IGF2-induced Cox-2-mediated p-ERK1/2 pathway. Therefore, we speculate that COX-2 in this WT microenvironment may drive the inflammation and upregulate the aforementioned downstream targets.

Possible therapeutic targets in Wilms tumors

On the basis of the above evidences, it was found that WTs have highly inflammatory microenvironment, which further provides a link for the inflammatory etiology of cancer. The overexpression of different inflammatory markers provides a rationale for their use in the prevention and treatment of cancer. More specifically, our observation (19) strongly supports the therapeutic value of blocking COX-2 in WTs. The overexpression of inflammatory markers in tumors, in particular COX-2, has provided a rationale for their targeting in prevention and treatment of many cancers (55–59) by COX-2-specific inhibitors alone (60, 61) or in combination

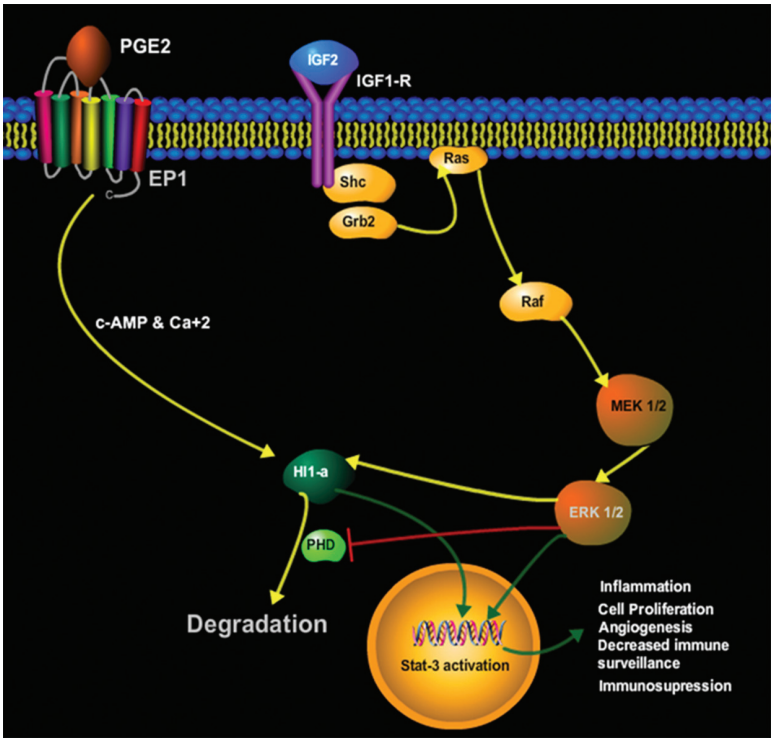

Figure 1. Possible mechanism/mechanisms responsible for COX-2 pathway activation: COX-2 activation of HIF-1 can also occur through hypoxia-dependent by PGE2 or hypoxia-independent mechanisms with the involvement of p-ERK1/2. HIF-1 can also directly upregulate the expression of COX-2 during hypoxia and thus form a feedback loop to continually activate the COX-2 pathway leading to activation. Hence, IGF2 affects the inflammation, hyperproliferation, and angiogenesis in WTs by IGF2-induced COX-2-mediated p-ERK1/2 pathway.

with other inhibitors (62). COX-2 inhibition may serve as an appropriate target for therapeutic intervention because all the downstream targets of COX-2 pathway components may be controlled or inhibited too. Also, COX-2 blockade may be effective in WT therapy owing to the inhibitory effect of COX-2 inhibitors in controlling the immune cell infiltration and tumor-promoting angiogenesis, thereby controlling tumor growth. Elucidating the molecular basis for the accumulation of the different inflammatory protein markers in tumors requires further in-depth study and warrants further investigation of this COX-2-mediated pathway.

Conclusions

The colocalization of TAMs in the tumor stroma along with COX-2 and its pathway components, such as HIF-1 and p-ERK1/2, suggests a functional association of TAM

infiltration with the overexpression of these markers and vice versa in WTs and demonstrate the existence of a highly inflammatory microenvironment in this cancer. The overexpression of inflammatory marker COX-2 has provided a rationale for their targeting COX-2 pathway using COX-2-specific inhibitors alone or in combination with other inhibitors, which may be effective in treating this childhood cancer.

Conflict of Interests

The author declares no potential conflicts of interest with respect to research, authorship and/or publication of this article.

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