

PROSTAGLANDIN E2 IN THE TUMOR MICROENVIRONMENT: A CONVOLUTED AFFAIR MEDIATED BY EP RECEPTORS 2 AND 4

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ABSTRACT

Prostaglandin E2 (PGE2) plays a pivotal role in modulating the tumor microenvironment (TME) and promoting tumorigenesis through complex signaling pathways mediated primarily by EP2 and EP4 receptors. Elevated levels of PGE2, driven by the upregulation of cyclooxygenase-2 (COX-2) in tumors, activate EP2 and EP4 receptors, which are involved in key processes such as cancer cell proliferation, survival, immune suppression, angiogenesis, and metastasis. Through the activation of cAMP, PI3K/AKT, and MAPK/ERK pathways, EP2 and EP4 promote tumor growth by enhancing cell proliferation and inhibiting apoptosis. These receptors also play a crucial role in immune evasion, fostering an immunosuppressive environment by influencing regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and other immune components. Moreover, PGE2 signaling via EP2 and EP4 contributes to the formation of new blood vessels and facilitates metastasis by promoting epithelial-mesenchymal transition (EMT). Due to their pro-tumorigenic roles, EP2 and EP4 receptors have emerged as promising therapeutic targets. The inhibition of these receptors, either alone or in combination with other treatments such as immune checkpoint blockade, holds potential for enhancing anti-cancer therapies. However, challenges such as resistance mechanisms and identifying suitable biomarkers for patient selection remain critical hurdles in optimizing EP2/EP4-targeted interventions.

1. INTRODUCTION

Prostaglandin E2 (PGE2): Overview of its Biosynthesis through the COX Pathway

Prostaglandin E2 (PGE2) is a bioactive lipid mediator derived from arachidonic acid, a polyunsaturated fatty acid found in the cell membrane. The biosynthesis of PGE2 is initiated when arachidonic acid is released from membrane phospholipids by the enzyme phospholipase A2. This is followed by the action of cyclooxygenase enzymes (COX-1 and COX-2), which convert arachidonic acid into prostaglandin H2 (PGH2), an unstable intermediate. Finally, PGE synthase catalyzes the conversion of PGH2 to PGE2.

COX-1 is constitutively expressed in many tissues and is involved in maintaining physiological functions such as gastric mucosa protection, whereas COX-2 is inducible and often upregulated during inflammatory processes, tissue injury, and in cancer. Overexpression of COX-2 in tumors leads to increased PGE2 production, which plays a significant role in promoting tumor growth and modifying the tumor microenvironment (TME).

Tumor Microenvironment (TME)

The tumor microenvironment (TME) refers to the complex and dynamic milieu surrounding cancer cells within a tumor. It consists of various cell types, extracellular molecules, and structural components that interact to regulate tumor progression and response to therapy. The TME is composed of:

- **Cancer cells:** The primary component of the TME, cancer cells undergo genetic mutations that drive uncontrolled proliferation and survival.
- **Immune cells:** Various immune cells, including T lymphocytes, macrophages, natural killer (NK) cells, and myeloid-derived suppressor cells (MDSCs), play dual roles in either supporting or inhibiting tumor growth. Tumors often manipulate immune cells to suppress anti-tumor immune responses, fostering immune evasion.
- **Stromal cells:** These include fibroblasts, particularly cancer-associated fibroblasts (CAFs), which secrete growth factors, cytokines, and extracellular matrix components that support cancer cell survival and metastasis.
- **Blood vessels:** Endothelial cells forming tumor-associated blood vessels are crucial for providing oxygen and nutrients to the growing tumor. This process of angiogenesis is often promoted by PGE2 via pro-angiogenic factors like vascular endothelial growth factor (VEGF).
- **Extracellular matrix (ECM):** The ECM is a network of proteins and carbohydrates that provide structural support. In cancer, ECM remodeling promotes tumor invasion and metastasis.

The TME not only provides structural support but also plays an active role in regulating cancer progression, immune response, and therapeutic resistance. PGE2, produced by both tumor cells and stromal components, serves as a critical signaling molecule that modifies the TME to favor tumor growth.

EP Receptors: Focus on the Four EP Receptors (EP1-EP4)

PGE2 exerts its biological effects through binding to EP receptors, a group of four G-protein-coupled receptors (GPCRs) known as EP1, EP2, EP3, and EP4. Each receptor has distinct downstream signaling pathways and functions, and the expression of these receptors can vary across different tissues, including tumors. The roles of the four receptors are as follows:

- **EP1 receptor:** Primarily associated with calcium mobilization, EP1 activation leads to increased intracellular calcium levels, contributing to processes such as pain perception and inflammation. Its role in cancer is less prominent compared to EP2 and EP4.
- **EP2 receptor:** EP2 is coupled to the Gs protein, which activates adenylyl cyclase, leading to increased levels of cyclic AMP (cAMP). The rise in cAMP activates protein kinase A (PKA) and downstream signaling pathways, which regulate cell proliferation, immune modulation, and angiogenesis. In the tumor microenvironment, EP2 has been shown to promote immunosuppression by enhancing the recruitment and activity of regulatory T cells (Tregs) and MDSCs, helping tumors evade immune detection.
- **EP3 receptor:** Unlike the other EP receptors, EP3 couples to **Gi proteins** and inhibits adenylyl cyclase, resulting in reduced cAMP levels. EP3 has been implicated in various physiological functions, including fever regulation and smooth muscle contraction, but its role in cancer is less defined.
- **EP4 receptor:** Similar to EP2, EP4 is also coupled to Gs proteins, leading to cAMP production and the activation of multiple downstream signaling cascades, including PI3K/AKT and MAPK/ERK pathways. EP4 has a well-established role in promoting tumor growth, immune suppression, and metastasis. In particular, EP4 signaling supports angiogenesis, tumor invasion, and the suppression of anti-tumor immune responses by influencing immune cell recruitment and activity within the TME.

Significance of EP2 and EP4 in Cancer Biology

Among the EP receptors, EP2 and EP4 are the most studied in the context of cancer biology due to their prominent roles in tumor promotion and immune modulation. Their signaling pathways overlap, particularly in their ability to activate the cAMP/PKA pathway, which drives a number of pro-tumorigenic processes. Through their interactions with immune cells, both receptors help tumors evade immune surveillance, making them attractive targets for cancer therapy. Additionally, their involvement in angiogenesis and metastasis further underscores the importance of EP2 and EP4 in cancer progression.

Therapeutic strategies aimed at **blocking EP2 and EP4** receptors or their downstream pathways have shown promise in preclinical cancer models, suggesting that inhibiting these receptors could be a valuable approach to disrupting the pro-tumorigenic effects of PGE2 and improving responses to other forms of cancer treatment, such as immunotherapy.

2. PGE2 BIOSYNTHESIS AND ITS REGULATION IN CANCER

Cyclooxygenase Pathway (COX-1 and COX-2): Role of COX-2 in Upregulated PGE2 Synthesis in Tumors

Prostaglandin E2 (PGE2) biosynthesis is initiated through the cyclooxygenase (COX) pathway, which converts arachidonic acid into prostaglandin intermediates. There are two key isoforms of cyclooxygenase:

- **COX-1:** This enzyme is constitutively expressed in most tissues and is responsible for maintaining normal physiological functions, such as gastric mucosal protection, renal blood flow regulation, and platelet function. It plays a less prominent role in cancer progression compared to COX-2.
- **COX-2:** Unlike COX-1, COX-2 is inducible and its expression is upregulated in response to various pro-inflammatory signals, growth factors, and oncogenic stimuli. COX-2 is overexpressed in many types of cancer, making it a critical factor in tumorigenesis. In the tumor microenvironment (TME), factors such as cytokines (e.g., IL-1 β , TNF- α), growth factors (e.g., EGF, VEGF), and oncogenic mutations (e.g., Ras) activate COX-2 expression. This leads to enhanced conversion of arachidonic acid into prostaglandin H2 (PGH2), a precursor for PGE2.

Once PGH2 is formed, it is further metabolized into PGE2 by prostaglandin synthases. COX-2-derived PGE2 is known to drive multiple hallmarks of cancer, including promoting tumor cell proliferation, evasion of apoptosis, angiogenesis, and immune suppression.

In many tumors, overexpression of COX-2 leads to sustained PGE2 production, which alters the tumor microenvironment (TME) in favor of cancer progression. Notably, PGE2 signaling through its EP receptors (particularly EP2 and EP4) fosters an immunosuppressive milieu and promotes processes such as invasion, migration, and metastasis.

PGE2 Metabolism: Enzymes Involved and their Role in Altering the TME

The synthesis of PGE2 involves several key enzymes. After the initial step mediated by COX-1 or COX-2, prostaglandin synthases (PGES), particularly microsomal prostaglandin synthase-1 (mPGES-1), play a crucial role in converting PGH2 to PGE2. The expression of mPGES-1 is often co-regulated with COX-2 and is upregulated in tumors, further driving PGE2 production.

PGE2 levels in the TME are tightly regulated not only by its synthesis but also by its degradation. 15-hydroxyprostaglandin dehydrogenase (15-PGDH) is the key enzyme responsible for PGE2 degradation, converting it to the inactive metabolite 15-keto-PGE2. However, 15-PGDH expression is frequently downregulated in cancer, resulting in the accumulation of PGE2 in the TME. The loss of 15-PGDH allows PGE2 to persist in the microenvironment, amplifying its pro-tumorigenic effects.

Effects on the Tumor Microenvironment (TME)

Elevated levels of PGE2 in the TME significantly alter its composition and function, fostering conditions conducive to tumor progression. The key effects of PGE2 in the TME include:

- 1. Immune Suppression:** PGE2 modulates immune cell activity within the TME to create an immunosuppressive environment. Through EP2 and EP4 receptor signaling, PGE2 recruits and activates regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), both of which inhibit anti-tumor immune responses. Additionally, PGE2 inhibits the activity of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, which are key players in tumor cell elimination.
- 2. Angiogenesis:** PGE2 promotes angiogenesis, the formation of new blood vessels, by inducing the expression of pro-angiogenic factors such as vascular endothelial growth factor (VEGF). This provides the growing tumor with an enhanced blood supply, facilitating its expansion and the dissemination of cancer cells.
- 3. Tumor Cell Proliferation and Survival:** PGE2 stimulates tumor cell proliferation and protects cancer cells from apoptosis by activating downstream signaling pathways, including the cAMP/PKA, PI3K/AKT, and MAPK/ERK pathways. These pathways promote cell survival, proliferation, and resistance to cell death, supporting tumor growth.
- 4. Invasion and Metastasis:** Through EP2 and EP4 receptor activation, PGE2 enhances epithelial-mesenchymal transition (EMT), a process by which cancer cells acquire increased motility and invasive capabilities. This facilitates metastasis, enabling cancer cells to migrate from the primary tumor site to distant organs.

3. THE ROLE OF EP2 AND EP4 IN TUMOR PROGRESSION

EP2 and EP4 Receptor Signaling Pathways

Prostaglandin E2 (PGE2) exerts its effects through four G-protein coupled receptors (GPCRs), namely EP1, EP2, EP3, and EP4. Among these, EP2 and EP4 play significant roles in cancer progression by activating critical intracellular signaling pathways.

- **G-protein Coupled Receptor Pathways:**

- Both EP2 and EP4 are coupled to Gas proteins, which activate adenylyl cyclase, leading to an increase in cyclic adenosine monophosphate (cAMP) levels.
- Elevated cAMP activates protein kinase A (PKA), which in turn phosphorylates downstream targets such as the CREB transcription factor. This drives the expression of genes that promote cell survival, proliferation, and immune suppression.
- Additionally, EP4 can activate PI3K/AKT and MAPK/ERK pathways through β -arrestin, independent of cAMP, further supporting its distinct pro-tumorigenic roles.

- **Differences Between EP2 and EP4 Signaling:**

- EP2 signaling is largely cAMP/PKA dependent, with its primary downstream effects occurring through CREB-mediated transcription and other cAMP-responsive elements.
- EP4, while also activating cAMP/PKA, is more versatile due to its ability to activate PI3K/AKT and MAPK/ERK pathways through β -arrestin scaffolding. This gives EP4 broader control over cell proliferation, migration, and survival.
- The PI3K/AKT pathway activated by EP4 enhances cellular survival and inhibits apoptosis, while the MAPK/ERK pathway promotes tumor cell proliferation and differentiation.

Pro-Tumorigenic Functions Mediated by EP2 and EP4

The PGE2-EP2/EP4 axis is central to numerous processes that support tumor progression, impacting cancer cell proliferation, survival, angiogenesis, immune evasion, and metastasis.

1. Cell Proliferation and Survival

- PGE2, through **EP2** and **EP4**, drives **cancer cell growth** by activating the **cAMP/PKA**, **PI3K/AKT**, and **MAPK/ERK** signaling cascades. These pathways increase the transcription of genes promoting **cell cycle progression** and survival.
- **EP4**, in particular, plays a key role in inhibiting apoptosis through the **AKT pathway**, which stabilizes anti-apoptotic proteins such as **Bcl-2** and inhibits pro-apoptotic factors like **BAD**. This gives cancer cells a survival advantage in the hostile tumor microenvironment.
- The **CREB** activation downstream of both receptors enhances the expression of survival and proliferative genes, further contributing to tumor growth.

2. Angiogenesis

- **EP2** and **EP4** both promote angiogenesis, primarily through the induction of **vascular endothelial growth factor (VEGF)**, a potent pro-angiogenic factor.
- Activation of the **MAPK/ERK** pathway by **EP4** enhances the expression of VEGF and other pro-angiogenic factors. This stimulates the growth of new blood vessels, providing the tumor with increased nutrients and oxygen supply to support expansion.
- **EP4** signaling also stimulates **endothelial cell migration and proliferation**, which are crucial steps in the formation of new vasculature.

3. Immune Evasion

- PGE2-EP2/EP4 signaling is a key mediator of **immune suppression** within the tumor microenvironment.
- Both receptors contribute to the recruitment and activation of **regulatory T cells (Tregs)** and **myeloid-derived suppressor cells (MDSCs)**, which are immunosuppressive cells that inhibit the activity of cytotoxic T cells and natural killer cells.
- **EP4** in particular is involved in the suppression of **dendritic cell maturation**, preventing effective antigen presentation and thereby diminishing anti-tumor immune responses.
- PGE2-mediated EP2/EP4 signaling also inhibits the production of **pro-inflammatory cytokines**, further shifting the TME towards an immunosuppressive state.

4. Metastasis and Invasion

- PGE2-EP2/EP4 signaling promotes **epithelial-mesenchymal transition (EMT)**, a process that allows cancer cells to acquire mesenchymal properties, including enhanced motility and invasiveness.
- Through **cAMP/PKA** and **PI3K/AKT** signaling, EP2 and EP4 induce the expression of **EMT transcription factors** like **Snail**, **Slug**, and **Twist**, which downregulate epithelial markers (e.g., E-cadherin) and upregulate mesenchymal markers (e.g., N-cadherin, vimentin).
- The resulting EMT enables cancer cells to detach from the primary tumor, invade surrounding tissues, and eventually metastasize to distant organs.
- Additionally, PGE2-EP4 signaling enhances **matrix metalloproteinase (MMP) production**, which facilitates the breakdown of the extracellular matrix, further aiding in cancer cell invasion.

4. INTERACTIONS WITH IMMUNE CELLS IN THE TUMOR MICROENVIRONMENT (TME)

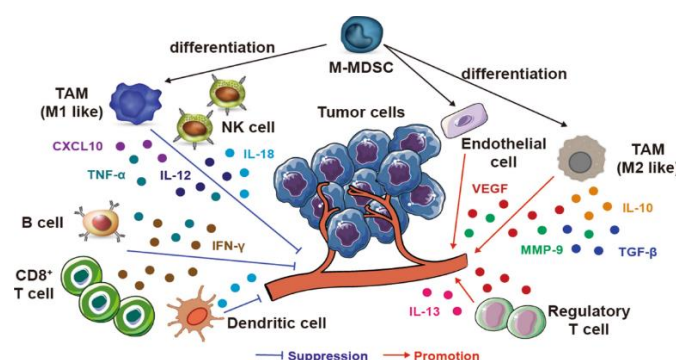


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Innate Immune Responses

PGE2 plays a crucial role in shaping the innate immune landscape within the tumor microenvironment by modulating the activity of key immune cells like macrophages, neutrophils, and natural killer (NK) cells. Through **EP2** and **EP4** receptors, PGE2 suppresses effective anti-tumor immunity, contributing to tumor immune evasion.

- **Macrophages:** PGE2 influences macrophage polarization, shifting them from a pro-inflammatory, anti-tumorigenic **M1 phenotype** to an immunosuppressive **M2 phenotype**. **M2 macrophages** promote tissue repair and tumor growth by secreting **immunosuppressive cytokines** (IL-10, TGF- β) and enhancing **angiogenesis**.
- **Neutrophils:** PGE2 can induce **neutrophil apoptosis** and suppress their activation. It also modulates the production of **reactive oxygen species (ROS)**, reducing their tumoricidal activity, thereby allowing tumor cells to evade neutrophil-mediated killing.
- **Natural Killer (NK) Cells:** PGE2 suppresses the cytotoxic activity of NK cells by impairing their ability to recognize and destroy tumor cells. The activation of **EP4** receptor signaling on NK cells reduces the production of pro-inflammatory cytokines like **IFN- γ** , limiting their anti-tumor responses.

Adaptive Immune Suppression

The immunosuppressive role of PGE2 in the adaptive immune response is primarily mediated by **EP2** and **EP4** signaling, which disrupts the balance between **regulatory T cells (Tregs)** and **effector T cells** and induces **T-cell anergy**.

- **T-cell Anergy:** PGE2-EP2/EP4 signaling suppresses the activation and proliferation of **cytotoxic T lymphocytes (CTLs)**, leading to a state of **T-cell anergy** (functional inactivation of T-cells), which reduces the immune system's ability to attack tumor cells.
- **Treg/effector T-cell Ratio:** PGE2 enhances the recruitment and expansion of **Treg cells** (which are immunosuppressive) while inhibiting **effector T-cell (Teff)** function, tipping the balance in favor of immune tolerance within the TME. **Tregs** secrete **IL-10** and **TGF- β** , creating an immunosuppressive microenvironment that further limits anti-tumor immunity.

5. EP2/EP4-TARGETED THERAPEUTIC APPROACHES

Pharmacological Inhibition

Targeting the **PGE2-EP2/EP4 axis** has emerged as a promising strategy for cancer therapy. Several **EP2/EP4 antagonists** and selective inhibitors are under investigation in both preclinical and clinical settings, aiming to disrupt the pro-tumorigenic signaling mediated by these receptors.

- **EP4 Antagonists:** **EP4-specific inhibitors**, such as **grapiprant** and **TPST-1495**, have shown potential in reducing tumor growth by blocking PGE2-mediated signaling pathways that support cell proliferation, immune suppression, and angiogenesis.
- **EP2/EP4 Dual Inhibitors:** Inhibitors targeting both **EP2** and **EP4** have demonstrated efficacy in **preclinical models**, where they suppress tumor progression by attenuating immune suppression and reducing metastatic potential.

Combination Therapies

Given the complex role of **EP2** and **EP4** in the tumor microenvironment, combining **EP2/EP4 inhibitors** with other therapeutic modalities has shown enhanced anti-tumor effects:

- **Immune Checkpoint Blockade:** Combining **EP2/EP4 antagonists** with immune checkpoint inhibitors (e.g., **anti-PD-1**, **anti-CTLA-4**) has shown synergistic effects in **restoring T-cell activity**, increasing anti-tumor immunity, and improving overall patient outcomes.
- **Chemotherapy and Radiotherapy:** **EP2/EP4 inhibitors** have been combined with traditional therapies such as chemotherapy and radiotherapy, which enhance tumor cell death. By reducing immune suppression, EP2/EP4 inhibitors may improve **tumor sensitivity** to these treatments.
- **Targeted Therapy:** Using **EP2/EP4 inhibitors** alongside targeted therapies like **VEGF inhibitors** (which block angiogenesis) has shown promise in **preclinical studies**, particularly in tumors that are highly dependent on angiogenesis for survival and metastasis.

6. CHALLENGES AND FUTURE DIRECTIONS

Resistance Mechanisms

One of the significant challenges in targeting the PGE2-EP2/EP4 axis is the potential for **tumor adaptation** and resistance. Tumors may **upregulate alternate pathways** that bypass PGE2 signaling or develop compensatory mechanisms through cross-talk with other **growth factor pathways** (e.g., **EGFR**, **VEGF**, or **TGF- β** signaling). This may limit the long-term efficacy of EP2/EP4-targeted therapies, necessitating the development of **combination strategies** or next-generation inhibitors.

Biomarkers

To improve patient selection for EP2/EP4-targeted therapies, there is a growing interest in identifying **biomarkers** that can predict response. Potential biomarkers include:

- **COX-2 expression** levels in tumors, as COX-2 overexpression correlates with increased PGE2 production.
- **EP receptor expression profiles** in different tumor types, which may guide the use of receptor-specific antagonists.
- **Immune cell infiltration patterns** (e.g., Treg levels, MDSCs), which could serve as indicators of a PGE2-driven immunosuppressive environment.

Novel EP Receptor Modulators

There is ongoing research to develop **next-generation EP receptor modulators** that are more selective and potent. These include:

- **Allosteric modulators** that target EP receptor activity without completely blocking receptor function.
- **Bi-specific inhibitors** that can simultaneously block **EP2/EP4** and additional tumorigenic pathways, offering a multi-pronged approach to combating cancer.
- **EP receptor degraders**, which could lead to the **downregulation of receptor levels** on the tumor cell surface, providing an alternative mechanism for reducing PGE2-driven signaling in the TME.

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