Prostaglandin E2 in the tumor microenvironment, a convoluted affair mediated by EP receptors 2 and 4

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Running title: PGE2 and its receptors in the tumor microenvironment

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Number of text pages: 55

Number of figures: 2

Number of tables: 2

Number of references: 436

Number of words in the abstract: 160
### Abbreviations:

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<th>Abbreviation</th>
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<tr>
<td>15-PGDH</td>
<td>15-hydroxyprostaglandin dehydrogenase</td>
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<td>AA</td>
<td>arachidonic acid</td>
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<td>ADCC</td>
<td>antibody-dependent cell-mediated cytotoxicity</td>
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<td>APCs</td>
<td>antigen presenting cells</td>
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<td>ARG1</td>
<td>arginase 1</td>
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<td>cAMP</td>
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<td>cDC1s</td>
<td>type 1 conventional DCs</td>
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<td>CIC</td>
<td>Cancer immunity cycle</td>
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<td>cNK</td>
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<td>COX</td>
<td>cyclooxygenase</td>
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<td>COX2 selective inhibitors</td>
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<td>cPGES</td>
<td>cytosolic PGE synthase</td>
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<td>cPLA2</td>
<td>cytosolic phospholipase A2</td>
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<td>CREB</td>
<td>cAMP-responsive element binding protein</td>
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<td>DCs</td>
<td>dendritic cells</td>
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<td>EP1</td>
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<td>EPRs</td>
<td>EP receptors</td>
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<tr>
<td>ERK</td>
<td>extracellular signal-regulated kinase</td>
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FasL  Fas ligand
GPCRs  G-protein-coupled receptors
Gs  Gs-stimulatory proteins
IFN-γ  interferon-γ
IL-1  interleukin 1
IL-10  interleukin 10
IL-2  interleukin 2
IL1-β  Interleukin-1β
LPS  lipopolysaccharide
MAPK  mitogen-activated protein kinase
MDSCs  myeloid-derived suppressor cells
MHC  major histocompatibility complex
mPGES  membrane-bound PGE synthase
NF-κB  nuclear factor-kappaB
NFAT  nuclear factor of activated T-cells
NKs  natural killer cells
NO  nitric oxide
NSAIDs  nonsteroidal anti-inflammatory drugs
NSCLC  non-small-cell lung carcinoma
OS  overall survival
PGE2  Prostaglandin E2
PGH2  prostaglandin H2
PGs  prostaglandins
PKA  protein kinase A
PKC  protein kinase C
PLC  phosphoinositide-phospholipase C
ROS  reactive oxygen species
TAMs  Tumor-associated macrophages
TANs  Tumor-associated neutrophils
TFs  transcription factors
TGF-β  transforming growth factor β
Th1  T-helper 1
Th17  T-helper 17
Th2  T-helper 2
TME  tumor microenvironment
TNF-α  tumor necrosis factor-α
TRAIL  TNF-related apoptosis-inducing ligand
Tregs  regulatory T-cells
VEGF  vascular endothelial growth factor
Abstract

The involvement of the prostaglandin E2 (PGE2) system in cancer progression has long been recognized. PGE2 functions as an autocrine and paracrine signaling molecule with pleiotropic effects in the human body. High levels of intratumoral PGE2 and overexpression of the key metabolic enzymes of PGE2 have been observed and suggested to contribute to tumor progression. This has been claimed for different types of solid tumors, including, but not limited to, lung, breast, and colon cancer. PGE2 has direct effects on tumor cells and angiogenesis that are known to promote tumor development. However, one of the main mechanisms behind PGE2 driving cancerogenesis is currently thought to be anchored in suppressed antitumor immunity, thus providing possible therapeutic targets to be used in cancer immunotherapies. EP2 and EP4, two receptors for PGE2, are emerging as being the most relevant for this purpose. This review aims to summarize the known roles of PGE2 in the immune system and its functions within the tumor microenvironment.
Significance statement

PGE2 has long been known to be a signaling molecule in cancer. Its presence in tumors has been repeatedly associated with disease progression. Elucidation of its effects on immunological components of the TME has highlighted the potential of PGE2 receptor antagonists in cancer treatment, particularly in combination with ICI therapeutics. Adjuvant treatment could increase the response rates and the efficacy of immune-based therapies.
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1 Introduction

The presence of leukocytes in tumors was first discovered in the 19th century. Rudolf Virchow (1821–1902) was among the first to report on the presence of immune cells in malignant tissues. Furthermore, he described chronic inflammation as a condition that could lead to cancer development (Piotrowski et al., 2020). Since then, an intricate interplay between the immune system and cancer progression on the one hand, and a causal link between inflammation and carcinogenesis on the other hand has been established. However, under the right conditions, the immune system can recognize and eliminate malignant cells. Modern cancer immunotherapies, such as immune checkpoint inhibitors (ICI) and adoptive cell therapies, can harness the immune system’s ability to recognize cancer cells and unleash an immune response to tumors.

The cancer-immunity cycle (CIC) must be allowed to initiate, proceed, and augment its induced immunological reactions by cycle repetition to make the body recognize, and build an immune response against, cancer (Figure 1) (Chen and Mellman, 2013; Pio et al., 2019). Chen and Mellman showed that the CIC starts with the release of cancer cell antigens (neoantigens) by malignant cells. Then, the released neoantigens can be detected and processed by antigen-presenting cells (APCs). The processed antigens are presented by APCs via major histocompatibility complex (MHC) in tumor-draining lymph nodes. Antigen presentation leads to the priming and activation of antigen-specific, effector T-cells. The activated T-cells are then trafficking to the tumor site and can infiltrate tumors to recognize and kill their target cancer cells. The disruption of the CIC at any given step results in the failure of the immune system to mount an effective
T-cell response to cancer cells (Chen and Mellman, 2013). Instead, the immune system allows tumor growth. This phenomenon is referred to as immune escape, a term describing a situation in which the immune system can no longer recognize and eliminate malignant cells (Dunn et al., 2002). Many factors must align favorably so that the immune system can successfully initiate and maintain immunosurveillance. It is not enough for cancer cells to merely release and present neoantigens that can be detected by APCs to achieve effective T-cell activation. The signaling context within the tumor microenvironment (TME) is crucial in determining whether the immune system will fight or tolerate malignant cells. Signaling within the TME can come from intercellular interactions, such as the interactions between immune checkpoints (e.g. PD-L1:PD-1) (Sharma et al., 2017). Furthermore, it can come from soluble factors produced by the cellular components of the TME. Signaling molecules, such as proinflammatory cytokines and lipid-derived mediators, contribute significantly to shaping the immunogenicity of tumors. T-cell responses can be directly and indirectly modulated by the signaling pathways activated by these molecules (Binnewies et al., 2018). The TME, as a sum of regulatory factors, acts as a determinant of the cancer immune response, and depending on its composition, it can suppress or promote antitumor immunity (Garner and de Visser, 2020).

The immunomodulatory effects of the TME are particularly important in the context of cancer immunotherapies. Even though this treatment approach has led to remarkable outcomes in several solid tumors, many patients remain unresponsive to these types of treatment (Topalian et al., 2012; Melief et al., 2015). For example, in the case of ICI in non-small-cell lung carcinoma (NSCLC), less than 45% of patients react favorably to
single treatment (Brahmer et al., 2012; Walsh and Soo, 2020). Immunotherapy-resistant tumors can have different characteristics, such as a lack of lymphocyte infiltration (cold tumors) or suppressed lymphocyte activation (immunosuppressed tumors) (Galon and Bruni, 2019). The causes for all of these can be most likely found within the TME. Nonresponsive patients could be sensitized by identifying key modulators within the TME and appropriate adjuvant therapies that reverse or circumvent their effects, thereby optimizing cancer immunotherapies.

2 Prostaglandin E2 (PGE2), a long-known cancer-promoting culprit

Prostaglandins (PGs) are lipid mediators belonging to the eicosanoid group. They serve as autocrine and paracrine signaling molecules and are involved in various biological processes. PGs are mainly derivatives of arachidonic acid (AA), and their biosynthesis starts with AA liberation from membrane phospholipids by cytosolic phospholipase A2 (cPLA2) (Figure 2). AA is then converted to PGG2 and subsequently to prostaglandin H2 (PGH2) by the cyclooxygenase (COX) enzyme. PGH2 is a substrate for different terminal (cell-specific) PG and thromboxane synthases. It can be converted to PGE2, PGD2, PGF2a, PGI2, and thromboxane A2 (Stack and DuBois, 2001). PGE2 is one of the most abundant PGs in the human body. This signaling molecule is an important mediator of many biological processes, such as pain, regulation of blood pressure and kidney function, gastrointestinal integrity, and fertility (Ricciotti and FitzGerald, 2011). However, PGE2 has also been shown to play a role in various biological processes associated with inflammation and cancer (Nakanishi and Rosenberg, 2013). PGE2 has
been associated with increased tumor angiogenesis, metastasis, apoptosis, cell cycle regulation, and immune suppression. High levels of PGE2 and/or its metabolic enzymes have been reported in many human solid tumors, including colon, stomach, lung, prostate, hepatocellular, and breast cancer, as well as melanoma (Koga et al., 1999; Soslow et al., 2000; Fang et al., 2003; Jang, 2004; Sandler and Dubinett, 2004; Khor et al., 2007; Panza et al., 2016; Karpisheh et al., 2019) The possible sources for PGE2 within the TME are many. Apart from tumor cells (Park et al., 2018; Gilardini Montani et al., 2021), macrophages (Esbona et al., 2018), dendritic cells (DCs) (Kim et al., 2013) fibroblasts (Schrey and Patel, 1995; Martey et al., 2004; Li et al., 2012, 2013), neutrophils (Hattar et al., 2014) and vascular endothelial cells (Mulligan et al., 2010) have been demonstrated to produce PGE2, which can increase dramatically in response to various immunological stimuli. These include interleukin (IL)-1, tumor necrosis factor-α (TNF-α), antigen-antibody complexes, and lipopolysaccharide (LPS) (Masucci et al., 2019).

Many studies have examined the relationship between PGE2 metabolism and cancer. Overall, a large body of evidence implicates PGE2 and its metabolic enzymes in cancer progression (Wang and DuBois, 2006; Nakanishi and Rosenberg, 2013; Kobayashi et al., 2018). High PGE2 levels have been demonstrated to promote tumor formation, progression, and metastasis in different cancer entities (Gomes et al., 2018) However, the mechanisms underlying the effects of PGE2 in the TME are not entirely elucidated. Over the past decades, extensive research has been conducted on this system and how it could be used to boost the efficacy of different cancer treatments. The focus has shifted from a metabolic approach, which reduced the levels of PGE2 present in the
TME, downstream to the receptors and signaling pathways activated by this molecule. This review aims to present a comprehensive overview of the PGE2 signaling system and its relevance in the TME and for cancer progression.

3 PGE2 synthesis and degradation

Given the established relationship between PGE2 levels and cancer progression, much research has focused on the enzymes responsible for its presence in tumors and in the body. COX is the key regulatory enzyme responsible for the biosynthesis of PGE2, of which there are two main isoforms, COX1 (PTGS1) and COX2 (PTGS2). The two isoforms differ from each other in important aspects, such as tissue distribution and expression patterns. COX1 is constitutively expressed in various cells and tissues, and its expression remains constant under most physiological and pathological conditions (Smith et al., 1996). COX1-derived PGs play a central role in many normal physiological processes. The activity of this enzyme is linked, for example, to renal function, gastric mucosal maintenance, stimulation of platelet aggregation, and vasoconstriction (Wang and DuBois, 2006). COX2 is the inducible isoform of the enzyme. It is normally absent in most cells and tissues. However, its expression is highly induced in response to proinflammatory cytokines, hormones, and tumor promoters. The expression of COX2 and subsequent levels of its final product PGE2 play a major role in inflammation and tumorigenesis.
The conversion of the COX product PGH2 to PGE2 can be catalyzed by three different prostaglandin E (PGE) synthases, including cytosolic PGE synthase (cPGES) (PTGES3) and two membrane-bound PGE synthases (mPGES1 (PTGES) and mPGES2 (PTGES2)) (Murakami et al., 2003). The expression of cPGES and mPGES2 is constitutive. mPGES1 is mainly an inducible isomerase. cPGES uses PGH2 produced by COX1, whereas mPGES1 uses COX2-derived PGH2 (Stack and DuBois, 2001). mPGES2 can metabolize PGH2 derived from the catalytic activity of both COX enzymes (Samuelsson et al., 2007). The preferential coupling between the COX and PGES enzymes might be due to colocalization. The cytosolic allocation of cPGES may facilitate its interaction with nearby COX1 in the endoplasmic reticulum, potentially favoring this interaction over COX2 (Tanioka et al., 2000). In the case of mPGES1, the concurrent presence of COX-2 and mPGES-1 within the same perinuclear membrane may facilitate their efficient functional interaction (Murakami et al., 2000).

Both inducible metabolic enzymes, COX2 and mPGES1, are overexpressed in NSCLC (Wolff et al., 1998; Yoshimatsu et al., 2001), colorectal cancer (Dimberg et al., 2001), breast cancer (Majumder et al., 2018) and other cancer entities (Takii et al., 2007; Cui et al., 2016; Garg et al., 2018; Fu et al., 2019; Zhu et al., 2020).

After its synthesis, PGE2 can exert its actions by acting on a group of G-protein-coupled receptors (GPCRs). The main GPCR subtypes that bind and respond to PGE2 include EP1, EP2, EP3, and EP4, with EP3 presenting multiple splicing isoforms. The EP subtypes show differences in signal transduction, tissue localization, and regulation of expression that will be discussed in detail in this review (Sugimoto and Narumiya, 2007). As is well known, the levels of a molecule present in a given tissue or cell are
dependent not only on its synthesis but also on its metabolic turnover. NAD$^+$-linked 15-hydroxyprostaglandin dehydrogenase (15-PGDH), which catalyzes PG oxidation, is the first and most important enzyme involved in PG catabolism. Its metabolic products have lower biological activities, making 15-PGDH the key enzyme for PG inactivation. The enzyme is widely present in various mammalian tissues, among which the lung is one of the most active tissues (Sun et al., 2021).

4 PGE2 in tumors

The amount of PGE2 present in tumors depends mainly on the expression and activity of the inducible enzymes COX2 and mPGES1 and on the rate at which it is degraded. COX2 overexpression has been detected in different cancer types, including colorectal (Kutchera et al., 1996; Fujita et al., 1998), prostate (Kirschenbaum et al., 2001), lung (Huang et al., 1998; Wolff et al., 1998), and breast cancer (Soslow et al., 2000). Inhibitors of this enzyme, such as nonsteroidal anti-inflammatory drugs (NSAIDs) or COX2 selective inhibitors (COXIBs), have been proven to have antitumorigenic effects. Studies conducted as early as 1988 have showed that the long-term administration of aspirin significantly reduced the risk of human colon cancer (Kune et al., 1988). Since then, cancer inhibitory effects of long-term administration of NSAIDs have been described in the context of different cancer types, such as esophagus (Jayaprakash et al., 2006), stomach (Farrow et al., 1998; Lindblad et al., 2005) and prostate cancer (Jacobs et al., 2005). In 2005, Harris et al. performed a meta-analysis of NSAIDs and cancer. They evaluated the literature results using epidemiological criteria and concluded that the daily intake of NSAIDs, primarily aspirin, leads to a cancer risk.
reduction of 63% for colon, 39% for breast, 36% for lung, and 39% for prostate cancer. Furthermore, significant risk reductions were observed for esophageal (73%), stomach (62%), and ovarian cancer (47%) (Harris et al., 2005). However, the prolonged use of NSAIDs and COXIBs is associated with adverse effects detrimental to patients' health, including cardiovascular risk and intracranial hemorrhage (Marsico et al., 2017; Huang et al., 2019).

Downstream of COX2, the synthase mPGES1 also plays a prominent role in pathophysiology (Samuelsson et al., 2007). Higher expression of this enzyme can be induced by growth factors and proinflammatory stimuli (Kim and Kim, 2022). Additionally, in vitro studies have revealed a functional interplay between mPGES-1 and COX-2, resulting in the effective synthesis of PGE2 in the context of inflammation (Murakami et al., 2000). This interplay between COX-2 and mPGES-1 has also been observed in various human cancer types, such as NSCLC (Yoshimatsu et al., 2001), squamous cell carcinoma of the larynx (Kawata et al., 2006) and colorectal cancer (Kamei et al., 2003). Furthermore, mPGES1 has been shown to be overexpressed in NSCLC (Yoshimatsu et al., 2001) and breast cancer (Mehrotra et al., 2006). Interestingly, the expression levels of mPGES1 do not always correlate with COX2 expression, indicating that the regulation of the two enzymes is not identical (Mehrotra et al., 2006; Samuelsson et al., 2007). In NSCLC cell lines, for example, IL-4 inhibited the formation of PGE2 predominantly via a decrease in COX2 expression, whereas the expression of mPGES1 was unaffected (Cui et al., 2006).

Nakanishi et al. have published a body of work focusing on the effects of mPGES-1 activity in colon cancer. Using genetic and carcinogen-induced colon cancer in vivo
models, they were able to show that the global genetic deletion of \textit{mPGES-1} effectively supresses intestinal tumorigenesis. However, their more recent work revealed that the targeted deletion of \textit{mPGES-1} in colon epithelia does not supress carcinogen-induced tumor development (Nakanishi and Rosenberg, 2021). This finding indicates that tumor cells are not necessarily the sole, or even the primary, source of PGE2 in the TME. Indeed, within a solid tumor many cell types may contribute to the generation of PGE2 (Kobayashi et al., 2018). Beyond tumor cells, an array of cell types including fibroblasts, endothelial cells, and certain types of immune cells, produce PGE2 via activation of COX2 and \textit{mPGES1} (Chang et al., 2004; Brecht et al., 2011; Monrad et al., 2011; Harizi, 2013; Weigert et al., 2018).

As previously mentioned, the quantity of PGE2 within tumors predominantly relies on its production by inducible enzymes COX2 and \textit{mPGES1}, as well as the pace of its degradation. Studies have indicated that, in NSCLC, overexpression of either COX2 or \textit{mPGES1} (Yoshimatsu et al., 2001) and underexpression of the degradation enzyme 15-PGDH (\textit{HPGD}) (Tai et al., 2007) are common. This has been shown to be true for most NSCLC cell lines (Yang et al., 2007) and patient tumor samples (Heighway et al., 2002; Li et al., 2014) at the mRNA and protein levels. Similar findings have been reported in the context of gastrointestinal cancers, wherein COX2 is markedly overexpressed while 15-PGDH remains scarcely detectable in colon cancers (Yan et al., 2004). The expression of COX2 and 15-PGDH seems to be reciprocally regulated (Tai et al., 2007; Li et al., 2014). Tong et al. conducted a study using A549 human lung adenocarcinoma cells and reported that the higher the induced expression of COX2, the lower the 15-PGDH expression. However, exogenous PGE2 did not induce a reduction
of 15-PGDH expression (Tong et al., 2006). Interleukin-1β (IL1-β) and TNF-α induce COX2 expression and simultaneously decrease the transcription of 15-PGDH (Otani et al., 2006; Tong et al., 2006; Thiel et al., 2009; Arima et al., 2018). The precise mechanisms underlying these effects remain incompletely understood, in the case of IL-1β, the reduced expression of 15-PGDH is, to some extent, attributed to the inhibition of 15-PGDH promoter activity (Thiel et al., 2009).

Co-overexpression of both COX2 and mPGES1 and reduced levels of 15-PGDH adversely affect tumor progression and overall survival (OS) of patients with NSCLC (Y-C Wu et al., 2010). 15-PGDH has been described as a tumor suppressor in NSCLC (Ding et al., 2005) and colon cancer (Backlund et al., 2005; Myung et al., 2006), among other types of cancer (Wolf et al., 2006; Kaliberova et al., 2009; Arima et al., 2019; Lee et al., 2019; Bu et al., 2022). A study conducted with A549 cells with adenovirus encoding 15-PGDH further supported its tumor-suppressive role. These cells showed slower tumor growth in athymic nude mice. Additionally, overexpression of this enzyme induced apoptosis of these cells in a p53-independent manner. Furthermore, the expression of 15-PGDH in A549 cells downregulates CD44, a receptor for hyaluronate that may promote tumor invasion (Ding et al., 2005). In pancreatic cancer, ablation of 15-PGDH in vivo dramatically promoted Aldh1-positive tumor cell expansion and KRAS-driven pancreatic tumorigenesis (Arima et al., 2019).

The prevailing consensus indicates that high levels of PGE2 and its anabolic enzymes in the TME of various types of solid tumors have pro-tumorigenic effects. Conversely, PGE2 has been reported to have antitumorigenic effects in lymphoma. An in vivo study
showed that knockdown of a gene responsible for one of the PGE2 receptors (Ptger4) in B-cell lymphoma markedly accelerated tumor dissemination, whereas Ptger4 overexpression provided significant protection (Murn et al., 2008). This phenomenon might be linked to a prolonged duration of signaling via the B-cell receptor in the absence of the EP4 receptor, which ultimately leads to higher proliferation. In the same study, Ptger4 was significantly downregulated in human B-cell lymphoma. Therefore, it was concluded that PGE2 signaling via EP4 in B cells functions as a tumor suppressor whose activity is regulated by PGE2 in the TME (Murn et al., 2008). Overall, research has demonstrated that PGE2 and its related enzymes wield substantial influence on tumor biology across a range of cancer types, with effects that are predominantly pro-tumorigenic.

5 The EP receptors (EPRs): expression, physiological functions, and their roles in cancer

PGE2 it is the most versatile of the PGs. Even though the pleiotropic activities of PGE2 are multiple and functionally opposed, the impact of any given signaling molecule on a biological system will ultimately depend on the activation of receptors that respond to this molecule, their distribution in the organism, and the signaling pathways they activate (Nakanishi and Rosenberg, 2013).

There are four known receptor subtypes that primarily respond to PGE2 as a ligand. They belong to the GPCR family and are designated EP1, EP2, EP3, and EP4, respectively. Each of these receptors is encoded by a distinct gene (Ptger1–4). It is
noteworthy that the EPRs are solely classified as such because they bind PGE2 with higher affinity than other prostanoids, as they are not closely related to each other based on their amino acid sequence. They display a low degree of homology, with most subtypes sharing only around 30% of their amino acid identity. Interestingly, some EPRs share higher percentage identity with other prostanoid receptors than among each other (Toh et al., 1995; Narumiya et al., 1999; Sugimoto and Narumiya, 2007). Each receptor is distinctively allocated in the body, and their expression levels are variable. Tissue and cellular localization for each subtype can also vary among species. Even within tissues, EPR subtypes show distinct cellular localization. The specific cellular expression of EPRs is also relevant in the context of cancer. EPR expression in cancer cells varies according to their cellular origin, and the effects of PGE2 on the cellular components of the TME will primarily depend on the EPR subtypes present on their surface and their activation. Furthermore, the different receptors display differences in binding affinity (Narumiya et al., 1999). Differential receptor affinity can lead to differential receptor activation of EPRs localized in the same cell type or tissues, which might be an important factor leading to the heterogeneity in responses to PGE2. Further complexity is added by the fact that, upon receptor binding, PGE2 stimulation can activate different G-proteins. Certain EPR subtypes couple to more than one G-protein and signal transduction pathway (Table 1). Therefore, the different effects of PGE2 on the body are a consequence of the diversity that EP subtypes exhibit with regard to biochemical properties (isoforms and ligand affinity), signal transduction (pathway activation), tissue and cellular localization, and regulation of receptor expression and availability (Coleman et al., 1994). The physiological and
pathophysiological functions of the different EPR subtypes have been extensively studied. Information on receptor function has been collected primarily using animal models deficient for each receptor and pharmacological studies using specific antagonists and agonists for the EPRs in animals and human tissues. In the literature, several reviews have extensively dealt with the signaling pathways and functions of the EPRs, including those by Narumiya et al., Sugimoto and Narumiya, and O’Callaghan and Houston. (Narumiya et al., 1999; Sugimoto and Narumiya, 2007; O’Callaghan and Houston, 2015). The upcoming sections summarize the existing information for each receptor in terms of distribution, expression, signaling pathways, regulation, and their documented roles in the context of cancer cells.

### 5.1 The EP1 receptor

EP1 receptor expression has only been found on specific organs of mice, such as the kidney, lung, stomach, small intestine, colon, spleen, skeletal muscle, brain (microglia), and testis (Funk et al., 1993; Watabe et al., 1993; Dey et al., 2006; Keene et al., 2009). In humans, EP1 is expressed in the myometrium (Senior et al., 1992), pulmonary veins (Norel et al., 2004), mast cells (Wang and Lau, 2006), colonic longitudinal muscle (Smid and Svensson, 2009), B cells (Nataraj et al., 2001) and keratinocytes (Konger et al., 2009; Woodward et al., 2011).

Of the four receptors, EP1 displays the lowest affinity for PGE2 (Table 1). The receptor is coupled to intracellular Ca^{2+} mobilization via Go_{q} protein (Watabe et al., 1993).
detail, ligand binding of EP1 activates this G-protein, and Gαq, in turn, activates phosphoinositide-phospholipase C (PLC), which generates the second messenger release of Ca^{2+} from intracellular stores and stimulates phosphorylation by protein kinase C (PKC). This pathway ultimately activates transcription factors (TFs), such as the nuclear factor of activated T-cells (NFAT), nuclear factor-kappaB (NF-κB), and mitogen-activated protein kinase (MAPK) (Sugimoto and Narumiya, 2007; O'Callaghan and Houston, 2015).

Initially described as responsible for smooth muscle constriction, the effects of EP1 activation are diverse. They range from neurological effects (neurotransmitter release), and mediation of stress response and pain perception (inflammatory thermal hyperalgesia) (Stock et al., 2001), to effects on the cardiovascular system (Rutkai et al., 2009; Avendaño et al., 2016) (EP1 promotes hypertension in mice), and the immune system (Nagamachi et al., 2007). As shown in a study of colon cancer in mice, EP1 receptor activation bolsters chemical carcinogenesis (Watanabe et al., 1999). In rats, pharmacological inhibition of EP1 has been shown to significantly reduce induced breast cancer incidence, multiplicity, and volume, and enhance cancer cell apoptosis (Kawamori et al., 2001). Studies showed that the activation of EP1 promotes cell migration and invasion in different cancers, thus contributing to carcinogenesis, aggressiveness, and tumor progression (Surh et al., 2011, 2012; Bai et al., 2013; Niu et al., 2019). The activation of EP1 has been demonstrated to promote cell migration and invasion (S-F Yang et al., 2010; Zhang et al., 2014). It also leads to better tumor cell adaptation to hypoxia (Kaidi et al., 2006; Kim et al., 2011). EP1 receptor expression has been reported for various kinds of tumor cells, including breast cancer (Thorat et al.,
2008), hepatocellular carcinoma (Breinig et al., 2008; Bai et al., 2013), and colon cancer (Gustafsson et al., 2007). The antagonism of the EP1 receptor was demonstrated to hinder tumor development in mouse models of breast cancer (Kawamori et al., 2001) and melanoma (Tober et al., 2006). An in vivo study using a colon cancer model showed that EP1 antagonism reduced Fas ligand (FasL) expression. FasL expression has been shown to be needed by cancer cells for optimal growth. Furthermore, EP1 antagonism attenuated tumor-induced immune suppression (O'Callaghan et al., 2013), the immune cell populations affected by the treatment will be further discussed in section 7.

Overall, the EP1 receptor displays distinctive expression patterns in both mice and humans. It displays the lowest affinity for PGE2 and its activation triggers a range of physiological effects, including cardiovascular modulation, and immune system regulation. EP1 activation boosts carcinogenesis in colon cancer models and has shown diverse impacts on cancer cell migration, invasion, and tumor progression across various cancers. Additionally, EP1 receptor antagonism has demonstrated potential in hindering tumor development.

5.2 The EP2 receptor

EP2 receptors are the least abundant of the EPRs in mice (Dey et al., 2006). Northern blotting revealed that murine EP2 mRNA is expressed most abundantly in the uterus, followed by the spleen, lung, thymus, ileum, liver, and stomach (Katsuyama et al., 1995). For humans, EP2 mRNA was detected at some level in most tissues, with the
highest expression levels present in the small intestine. EP2 mRNA was detected at lower but significant levels in the lung, kidney, thymus, uterus, and brain. However, no expression was detected in the liver, heart, retina, or skeletal muscle (Bastien et al., 1994).

The major signaling pathway of EP2 receptors starts with the activation of $G_s$-stimulatory proteins ($G_\alpha_s$). The activation of this G-protein type leads to increased cyclic AMP (cAMP) levels in the cell via the activation of adenylate cyclase (Regan et al., 1994). Increased levels of cAMP then activate protein kinase A (PKA) (Fujino et al., 2005). PKA, in turn, activates different TFs and pathways, such as the cAMP-responsive element binding protein (CREB) (Fujino et al., 2005). EP2-activated $G_s$ can also activate the $\beta$-catenin pathway independently of cAMP via Axin (Castellone et al., 2005).

EP2 differs from all other PG receptors in that it does not undergo homologous desensitization (Malty et al., 2016). Consequently, EP2 can act over prolonged periods compared with other PG receptors. EP2 receptor activation can significantly induce the expression of proinflammatory factors, such as IL-1$\beta$, IL-6 (Merz et al., 2016), IL-23 (Sheibanie et al., 2004; Boniface et al., 2009), and CXCL1 (Zelenay et al., 2015), and decrease the levels of IL-12, IL-27 (Sheibanie et al., 2007), interferon-$\gamma$ (IFN-$\gamma$) (Lejeune et al., 2014), TNF-$\alpha$ (Meja et al., 1997; Kashmiry et al., 2018), and IL-2 (Chouaib et al., 1985). The activation of the $\beta$-catenin pathway via the EP2 receptor promotes tumor growth in colon cancer (Castellone et al., 2005) and increases transcription genes implicated in cancer, such as vascular endothelial growth factor (VEGF) (Fujino et al., 2002; O'Callaghan and Houston, 2015). Therefore, the activation of EP2 promotes
angiogenesis in tumors, with the deletion of EP2 impairing angiogenesis (Sales et al., 2004; Chang et al., 2005; Kamiyama et al., 2006). EP2 is abnormally expressed in various solid tumor cells, including colon (Hsu et al., 2017), prostate (Merz et al., 2016), and liver (Zang et al., 2017). The receptor has been shown to be involved in positive feedback regulation of COX2 expression. Thus, the activation of EP2 leads to even higher levels of PGE2 being present in the TME and amplification of PGE2 signaling (Obermajer et al., 2011). The activation of EP2 directly suppresses immune cell functions. The effects mediated by the EP2 signaling pathway on different immune cell groups will be further revisited in this review. Also, a thorough overview of the involvement of the EP2 receptor in cancer was published by Sun and Li in 2018 (Sun and Li, 2018).

In summary, the EP2 receptors exhibit lower abundance in mice and varied tissue expression in humans. Their primary activation pathway initiates a signaling cascade involving cyclic AMP and protein kinase A. Their unique resistance to desensitization allows for prolonged effects. EP2 activation influences proinflammatory factors and cytokine levels, promoting angiogenesis and supporting tumor growth. Abnormal expression in tumor cells, involvement in COX2 regulation, and immune cell suppression contribute to their role in cancer.

5.3 The EP3 receptor

Along with EP4, EP3 is the most widely expressed receptor of the EP family. Studies measuring mRNA expression showed that, in mice, EP3 receptors are expressed in almost all tissues. The receptor has also been shown to be widely distributed in
humans, with one study showing that EP3 mRNA is most abundantly present in the kidney, pancreas, and uterus (Kotani et al., 1995). Multiple species express different isoforms of the EP3 receptor (Negishi et al., 1995). All these isoforms are generated by alternative splicing at the C-terminus of the protein. For humans, eight different isoforms of the EP3 receptor (Adam et al., 1994; Kotani et al., 1995; Schmid et al., 1995; Bilson et al., 2004), while for mice, only three have been published (Irie et al., 1993; Sugimoto et al., 1993). EP3 and EP4 have a higher affinity for PGE2 than EP2 and EP1. Human EP3 has the lowest dissociation constant of all human EPRs (Narumiya et al., 1999; Abramovitz et al., 2000; Dey et al., 2006).

Multiple isoforms of EP3 have almost identical ligand binding properties. However, they differ in their subsequent signaling pathways, rate of desensitization, and constitutive activity (Kotani et al., 1995). Different EP3 isoforms can be linked to different G-proteins, leading to functional differences. In mice, the α and β isoforms of EP3 are solely coupled to inhibit adenylate cyclase via G_i. The γ isoform can be coupled to both G_s and G_i, thus causing the inhibition of adenylate cyclase at low concentrations of PGE2 and stimulation at high concentrations (Namba et al., 1993; Sugimoto et al., 1993). The major signaling pathway of the EP3 receptor is the inhibition of adenylate cyclase via G_q and activation of the Ras/Raf and MAPK signaling pathways (Woodward et al., 2011). Nevertheless, some EP3 isoforms also activate G_q (Yamaoka et al., 2009); in T-cells and other inflammatory cells, the activation of G_q-coupled receptors leads to increased intracellular calcium levels, thus leading to immune cell activation (Tilley et al., 2001). PGE2 mediates fever generation in response to both exogenous and endogenous pyrogens by acting on EP3 (Ushikubi et al., 1998). The activity of this
receptor protein is also linked, among other things, to vasoconstriction (Norel et al., 2004), platelet aggregation (Mawhin et al., 2015), suppression of allergic inflammation (Kunikata et al., 2005), and cough induction (Maher et al., 2011).

In the context of cancer, EP3 mediates angiogenesis associated with tumor development and chronic inflammation (Amano et al., 2003, 2009). An *in vitro* study using the human lung cancer cell line A549 showed that PGE2 activates Src signaling via EP3. The activation of this pathway was shown to stimulate cell growth (Yamaki et al., 2004). Another study showed that EP3 inhibition led to reduced cell viability, migration, and invasion. It also led to higher apoptosis rates in A549 cells (Li et al., 2018). These effects were shown to be associated with TGF-β/Smad signaling, suggesting that an EP3-TGF-β/Smad signaling axis is involved in the regulation of lung cancer cells.

In short, EP3 is widely distributed and expressed in various isoforms that differ from each other in their biochemical properties. It couples to different G-proteins, leading to diverse effects and physiological functions, including immune activation and inflammation. In cancer, EP3 promotes angiogenesis and affects lung cancer cell growth, migration, and apoptosis via TGF-β/Smad signaling.

### 5.4 The EP4 receptor

Studies measuring mRNA expression showed that, in mice, EP4 receptors are expressed in almost all tissues. EP4 is widely expressed in humans as well (Sugimoto and Narumiya, 2007). The reported functions for EP4 include cardiomyocyte hypertrophy (Qian Jian-Yong et al., 2008), vasodilatation (Baxter et al., 1995; Davis et
al., 2004), and angiogenesis (Xin et al., 2012; Majumder et al., 2014). EP4 is upregulated in different cancer entities and contributes to their pathology. These include lung, colon, and breast cancer (Reader et al., 2011; Majumder et al., 2018; Take et al., 2020).

EP4 and EP2 receptors act redundantly in some processes, with both receptors activating G\(\alpha_s\) and increasing cAMP within cells (Watabe et al., 1993; Regan et al., 1994). However, there are fundamental differences between the two receptors, not only structurally but also in terms of pathway activation and regulation. While the binding of PGE2 to EP2 activates a positive feedback loop, EP4 receptors are found to undergo rapid ligand-induced desensitization upon PGE2 binding, making the signal transduction capacities of EP4 less potent than those of EP2. This desensitization may partially be linked to the rapid internalization of EP4 in response to PGE2 binding, a characteristic not shared by EP2 (Desai et al., 2000). However, there are reports of positive feedback in PGE2 secretion, as COX2 expression is induced in cultured mouse podocytes following EP4 stimulation. Furthermore, multiple known cAMP-dependent signaling pathways can be triggered by the EP4, including those involving PKA (Xu et al., 2018), AMPK (Faour et al., 2008), and Epac (Bos, 2003). These might act in concert or in an alternating manner to mediate the G\(\alpha_s\)-dependent effects of the EP4 receptor (Konya et al., 2013). EP4 can also be linked to other noncanonical pathways, such as the PI3K/AKT and the extracellular signal-regulated kinase (ERK). The two pathways promote cell survival, migration, and proliferation, respectively. The activation of the EP4-PI3K/AKT has been proposed to regulate the migration and metastasis of colon carcinoma cells (Buchanan et al., 2006). Konya et al. (Konya et al., 2013) and
Yokoyama et al. thoroughly reviewed the different signaling pathways linked to EP4 (Yokoyama et al., 2013).

In conclusion, EP4 is widely distributed in humans and mice and involved in various physiological functions such as vasodilatation and angiogenesis. While EP4 shows some signal transductions overlap with EP2, it is less sensitive to PGE2. Its upregulation in various cancer cell types contributes to their progression. Furthermore, Take et al. provided a comprehensive overview of the involvement of the EP4 receptor in cancer in 2020 that outlines how PGE2/EP4 signal inhibition can help restore the CIC (Take et al., 2020). The known ways that EP4 activation via PGE2 in the TME affects immune cells of will be discussed further in this review.

6 EPRs and immune cells in the TME - How PGE2 affects elements of the CIC

Tumor tissue is composed of tumor cells, the surrounding stroma and immune cells. It also contains various types of signaling molecules. All these components collectively contribute to the TME. The composition of the TME of any given tumor is a determinant of cancer progression. Studies focusing on PGE2 and its metabolism have highlighted the relevance of PGE2 for tumor development. Further studies have shown that PGE2 modulates not only malignant cells but also other important factors of tumor development, such as angiogenesis and antitumor immunity, making PGE2 an important modulator of the TME.
PGE2 promotes an immunosuppressed TME by directly or indirectly affecting diverse immune cell groups, making it a key disruptor of the CIC. Elucidating how PGE2 affects immune cell populations that infiltrate tumors is therefore key to understanding the mechanisms behind the effects of PGE2 on cancer. The effects that PGE2 in the TME has on immune cells, which may disrupt the CIC, have been the subject of multiple investigations (Mizuno et al., 2019; Finetti et al., 2020). In different cancer entities, PGE2 has direct and indirect effects on many cellular components of the immune system. The effects of PGE2 on immune cells are mediated mainly by EP2 and EP4. Understanding how the PGE2-EP2/4 axis in the TME works (cellular functions, EPR expression on immune cells, and PGE2 abundance in the tumor) could lead to the discovery of new therapeutic targets and an improvement of immune therapy outcomes for patients with cancer.

The CIC model serves as a valuable framework for understanding the immune system’s response to cancer and the potential applications of immunotherapy. As previously highlighted in the Introduction of this review, the CIC delineates the crucial steps required for the immune system to identify, target, and eliminate cancer cells. The effectiveness of the CIC is contingent upon the presence and functionality of various immune cell types. The following sections summarize the roles of diverse immune cell populations within the tumor microenvironment (TME), explore the impact of PGE2 on these cells, and consider how these interactions can influence the progression of the CIC. Additionally, Table 2 provides a comprehensive overview of different immune cell types, their known responses to PGE2, and the PGE2 receptors involved in these responses.
### 6.1 CD8⁺ T-cells

CD8⁺ T-cells are cytotoxic T-cells. Once activated into their effector state, these cells can target and kill other cells in an antigen-dependent manner. The absence of effector CD8⁺ T-cells in the TME correlates with poor prognosis for clinical outcomes in both human cancer and animal models (Lohneis et al., 2017; Peranzoni et al., 2018; Take et al., 2020). Thus, a successful CD8⁺ T-cell response is the pinnacle of immune generation via the CIC. (Chen and Mellman, 2013). For cytotoxic T-cells to be able to fulfill their role within the TME, all the steps of the CIC need to be successful and the T-cells fully functional.

It is well known that the activation of PD-1/PD-L1 signaling in tumors can inhibit T-cell function and weaken a full immune response (Farhood et al., 2019). A human lung cancer study using isolated CD8⁺ T-cells showed that PD-1 expression in these cells positively correlates with PGE2 signaling via the EP2 and EP4 receptors (Wang et al., 2018). Thus, the activation of EP2 and EP4 signaling pathways on CD8⁺ T-cells by PGE2 in the TME may induce the expression of PD-1, which in turn, leads to immune tolerance.

In an in vivo model for diabetes, PGE2 attenuated T-cell receptor-induced IFN-γ release, an effect that was mediated by EP2 and EP4 via intracellular cAMP production (Ganapathy et al., 2000). A later study showed that pharmacological inhibition of EP4 led to an increase in IFN-γ production by CD8⁺ T cells of intestinal adenomas. (Wei et al., 2022) IFN-γ enhances CD8⁺ T-cell cytotoxicity(Bhat et al., 2017). Furthermore, EP1 antagonism in a colon cancer model led to an increase cytotoxic activity of CD8⁺ T-cells
isolated from the spleen of tumor bearing mice (O’Callaghan et al., 2013). Another mechanism by which PGE2 can suppress cytotoxic function of CD8$^+$ T-cells is by upregulating the expression of inhibitory receptors like the CD94/NKG2A heterodimer (Zeddou et al., 2005). PGE2 can also indirectly inhibit the cytotoxic activity of effector CD8$^+$ T-cells by activating immune-suppressive cell types in the TME, such as Tregs and M2 macrophages (Albu et al., 2017; Saha et al., 2020; Xun et al., 2021). Moreover, PGE2 inhibits CD8$^+$ T-cell proliferation by promoting replicative senescence (Chou et al., 2014). Replicative senescence refers to a cellular condition characterized by cellular dysfunction and irreversible cell cycle arrest, accompanied by significant alterations in gene expression. This state can contribute to compromised immune responses in contexts like aging and chronic infections. Chou et al. demonstrated in vitro that chronic activation of CD8$^+$ T-cells, in the presence of PGE2, triggers replicative senescence. These T-cells subsequently display hallmark features of senescence, including diminished proliferative capacity, telomerase activity loss, reduced CD28 expression, and the suppression of cytokine expression (IL-2, IFN-$\gamma$, TNF-$\alpha$). Given that these cells are dysfunctional, they are unable to recognize and kill cancer cells (Huff et al., 2019).

In short, the proper function of CD8$^+$ T-cells is needed for the culmination of the CIC and PGE2 has been described to directly and indirectly inhibit the proliferation and cytotoxic functions of these cells in the TME via different mechanisms.

### 6.2 CD4$^+$ T-cells
CD4+ T-cells are crucial elements of the adaptive immune response. Naïve CD4+ T-cells can differentiate into specific subtypes after activation via antigen presentation. These subtypes include the T-helper 1 (Th1), T-helper 2 (Th2), and T-helper 17 (Th17) subsets, as well as regulatory T-cells (Tregs) (Luckheeram et al., 2012). The maturation and differentiation of different lineages are driven by chemokines and by their interactions with APCs (Mosmann and Coffman, 1989; Zhu et al., 2010). Subtypes differ from one another in their patterns of cytokine production, patterns of cell surface molecules, and function.

The different CD4+ T-cell subsets play different roles in the TME. Th1 cells are characterized by secretion of IFN-γ and TNF-α. They are primarily responsible for activating and regulating cytotoxic T-cells (Knutson and Disis, 2005). Additionally, they can directly kill tumor cells via the release of cytokines that activate death receptors on the tumor cell surface. (Knutson and Disis, 2005; Basu et al., 2021) In the context of the CIC, Th1 cells are involved in the priming and activation of cytotoxic T-cells via cell to cell interactions, as well as in cancer antigen presentation (Chen and Mellman, 2013; Borst et al., 2018).

Th2 have been mostly considered to be pro-tumorigenic. These cells often secrete cytokines that hinder the differentiation and activity of Th1 cells (Matsuo et al., 2021). Consequently, Th2 cells within the TME might indirectly deter anti-tumor immunity. For instance, Th2 cells generate IL-10 (Basu et al., 2021). This cytokine can dampen Th1-driven immune responses, primarily by downregulating Th1 cytokine function and APCs. However, a recent report showed in in vivo models for cancer of the colon and pancreas
that Th2 cells can promote anti-tumorigenic responses from macrophages and eosinophils (Jacenik et al., 2023).

Both pro-tumorigenic and anti-tumorigenic functions have also been ascribed to Th17 cells (Zou and Restifo, 2010). On the one hand, Th17 cells produce IL-17A. This cytokine induces the production of angiogenic factors (Numasaki et al., 2003). It also enhances the infiltration of myeloid-derived suppressor cells (MDSCs) into tumor tissues and activates their immunosuppressive activity (He et al., 2010). On the other hand, in some types of cancer, Th17 have been shown to recruit T-cells and DCs to the TME (Martin-Orozco et al., 2009), boost NK cell function (Al Omar et al., 2013) and elicit tumor-specific cytotoxic T-cell responses (Pan et al., 2023).

Tregs play a significant role in facilitating tumor evasion from immunosurveillance (Ohue and Nishikawa, 2019). They hinder the activation and expansion of effector T-cells and DCs (Huang et al., 2004; Tanaka and Sakaguchi, 2017; Li et al., 2020). What is more, Tregs release various immunosuppressive cytokines that inhibit the activation of tumor-specific effector T-cells or promote their exhaustion (Sawant et al., 2019; Matsuo et al., 2021). Moreover, Tregs impede DC activation and block vital costimulatory signals from DCs required for the initiation of tumor-specific T-cell reactions (Liang et al., 2008).

The expression of EP2, EP3, and EP4 has been proven in human CD4\(^+\) T-cells at an mRNA level (Okano et al., 2006). PGE2, as a cAMP-elevating agent, plays a suppressive role in Th1 activation and proliferation (Smith, Steiner, and Parker, 1971; Smith, Steiner, Newberry, et al., 1971; Sreeramkumar et al., 2012). PGE2 can also reduce the production of Th1-associated cytokines such as IFN-\(\gamma\), causing a switch from a Th1 to a Th2 cytokine secretion profile (Benbernou et al., 1997; Demeure et al.,...
What is more, in vitro, PGE2 polarizes CD4+ T-cells toward Th2 development via indoleamine 2,3-dioxygenase overexpression in DCs (Shimabukuro-Vornhagen et al., 2013). However, more recent reports contradict this notion and suggest that PGE2 could induce Th1 differentiation under certain conditions via the EP4 receptor. Yao et al. showed that PGE2 facilitated IL-12-induced Th1 differentiation at nanomolar concentrations under strengthened T-cell receptor stimulation. This differentiation occurred via the PI3K pathway, not via the cAMP pathway expansion. This could mean that the role of PGE2 in Th1 differentiation could be concentration-dependent (Yao et al., 2009; Sakata et al., 2010b; a). They also demonstrated, using models for autoimmune diseases, that PGE2 signaling through the EP4 receptor promotes Th17 expansion, a phenomenon confirmed in other study settings (Chen et al., 2010; Gagliardi et al., 2010; Sakata et al., 2010a; Yokoyama et al., 2013). In NSCLC, PGE2 induced expression of Foxp3, and increased Treg activity was observed. Foxp3 gene expression was significantly reduced in the absence of EP4 and eliminated in the absence of the EP2 receptor. Furthermore, COX2 inhibition reduced CD4+ CD25+ Treg cell frequency and activity, attenuated Foxp3 expression in tumor-infiltrating lymphocytes, and decreased tumor burden in vivo (Sharma et al., 2005). Moreover, EP1 antagonism in an in vivo colon cancer model led to a reduced infiltration by Tregs (O’Callaghan et al., 2013). Certain Treg subpopulations produce PGE2 in vitro, suggesting that PGE2, through autocrine and paracrine effects, is responsible for both, Treg phenotypes and the mechanism used by these cells to suppress CD8+ effector T-cells (Mahic et al., 2006). In mice, PGE2 regulates Foxp3 via EP2 and EP4. This has
been demonstrated *in vitro* by cultivating Tregs isolated from the spleen of EP2 or EP4 and in knockout mice and by EP2/EP4 antagonist treatment (Mahic et al., 2006). 

In summary, some subtypes of CD4\(^+\) T-cells are directly involved in certain steps of the CIC like antigen presentation, the priming and activation of CD8\(^+\) T-cells and even the release of tumor antigens. Other subtypes facilitate immune evasion by modulating the function of other key immune cell populations. PGE2 can modulate CD4\(^+\) T-cell responses, with most of the described effects leading to disruptions of the CIC.

### 6.3 B-cells

The primary function of B-cells within the TME and in the context of the CIC is to engage in antigen-specific interactions with T-cells that are crucial to their protective role. Besides their antigen-presenting function, they also secrete antibodies and various cytokines. However, tumor-infiltrating B-cells can differentiate into different subtypes under the influence of the TME. They can be pro- or antitumorigenic based on their differentiation state (Guy et al., 2016; Horii and Matsushita, 2021).

Regarding EPR expression, it is known that splenic B-cells of mice express EP1, EP2, and EP4, but not EP3, as shown via mRNA expression measurements (Nataraj et al., 2001). However, the effects of PGE2 on B-cells partially depend on their maturation stage. In mice, PGE2 has inhibitory effects on B-cell development. It suppresses their proliferation and induces their apoptosis via EP4 (Brown et al., 1992; Prijatelj et al., 2011). In the human B-cell line WEHI 231,a model for immature B cells, PGE2-EP4 signaling was shown to promote B-cell receptor-induced G0/G1 arrest in cAMP and NF-
κB dependent pathways (Prijatelj et al., 2012). Furthermore, in vitro experiments with human cells showed that the proliferation and differentiation of B-cells coming from peripheral blood are suppressed by PGE2 (Simkin et al., 1987). A later study in the context of B-cell lymphoma confirmed the inhibition of proliferation and showed that it is mediated by EP4 (Murn et al., 2008). Mature human B-cells are unaffected by PGE2-induced apoptosis and can even enhance their proliferation (Garrone et al., 1994). However, PGE2 inhibits B-cell activation (Jelinek et al., 1985). When LPS- and IL-4-activated B-cells are treated with PGE2, this inhibits their enlargement and the hyperexpression of MHC-II and FcεRII (Fedyk and Phipps, 1996). Another study showed that signaling via EP4 potently repressed the expression of multiple MHC components (Murn et al., 2008). PGE2 also stimulates the production of IgG1 and IgE and diminishes IgM production (Fedyk and Phipps, 1996). A study in the context of Asthma showed that the way PGE2 promotes IgE production in vivo is via EP2 (Gao et al., 2016). In the context of cancer, IgE may play a favorable role in antitumor immunity, among others by promoting M1 macrophage polarization. (McCraw et al., 2021).

Overall, B-cells within the TME and have a multifaceted role that can lead to pro- or anti-tumorigenic effects. Their primary function in the context of the CIC involves antigen-specific interactions with T-cells. However, they can also secrete antibodies and various cytokines. PGE2 can inhibit B-cell activation, reduce MHC expression, and modulate immunoglobulin production, but its effects on B-cells are highly dependent on their maturation stage.
6.4 Eosinophils

Both pro and anti-tumorigenic roles have been described for eosinophils in the TME. They can release chemokines like CCL5, CXCL9, and CXCL10 to attract CD8+ T-cells (Carretero et al., 2015). In vitro and in vivo experiments have shown that eosinophils directly kill tumor cells by releasing granular content, leading to the release of cancer cell antigens (Costain et al., 2001; Lucarini et al., 2017; Varricchi et al., 2017). Moreover, tumor-associated eosinophils have been demonstrated to facilitate the normalization of tumor blood vessels, leading to changes in the tumor that may favor the increase of T-cell infiltration and activity (Carretero et al., 2015). All these effects therefore contribute to the progression of the CIC. On the other hand, conflicting data exists about their effects on Tregs and macrophages. Some studies suggest eosinophils are associated with Treg depletion and promote M1-like macrophage phenotypes (Carretero et al., 2015). However, there are other studies that have reported that eosinophils lead to the recruitment of Tregs via CCL22 (Zaynagetdinov et al., 2015) and macrophage polarization towards the M2-like immunosuppressive phenotype via IL-13 (Kratochvill et al., 2015). Ultimately, the effects that eosinophils have on the TME may be determined by the stimuli they get from the TME (Simon et al., 2019).

For eosinophils in humans, the expression of EP4 and EP2 has been detected at an mRNA and protein level (Mita et al., 2002; Sturm et al., 2008; Luschnig-Schratl et al., 2011). EP4 and EP2 activation suppresses eosinophil responses such as chemotaxis and degranulation (Sturm et al., 2008; Luschnig-Schratl et al., 2011). Furthermore, Luschnig-Schratl et al. showed that the selective activation of EP4 resulted in inhibition
of intracellular Ca\(^{2+}\) release, reorganization of the cytoskeleton, and generation of reactive oxygen species (ROS). Additionally, EP4 receptor activation was found to impede CD11b upregulation, the activation and clustering of β2 integrins, and the shedding of L-selectin in eosinophils (Konya et al., 2011).

All in all, eosinophils in the TME exhibit both pro-tumorigenic and anti-tumorigenic functions that may have an influence on the progress of the CIC. They express EP4 and EP2 receptors, and their activation can suppress eosinophil activation and responses such as chemotaxis and degranulation.

### 6.5 Neutrophils

There are reports on both pro- and antitumorigenic functions of neutrophils within the TME (Que et al., 2022). On the one hand, neutrophils can contribute to the release of cancer cell antigens through various mechanisms. They possess cytotoxic capabilities that can be either antibody-dependent (Matlung et al., 2018) or mediated by ROS (Granot et al., 2011). They have also been demonstrated to mediate apoptosis via TNF-related apoptosis-inducing ligand (TRAIL) (Koga et al., 2004). Additionally, neutrophils can produce cytokines and chemokines that facilitate the recruitment and activation of CD8\(^{+}\) T-cells (Que et al., 2022). A study by Suttman et al. demonstrated \textit{in vivo} that the depletion of neutrophils significantly hindered T-cell trafficking and reduced the effectiveness of immunotherapy for bladder cancer (Suttmann et al., 2006).

On the other hand, neutrophils can play a substantial role in promoting immunosuppression (Coffelt et al., 2016). They have the capacity to induce CD8\(^{+}\) T-cell
apoptosis through a mechanism involving TNF-α pathway and nitric oxide (NO) (Michaeli et al., 2017). Moreover, tumor-associated neutrophils (TANs) can suppress CD8+ T-cell proliferation via arginase 1 (ARG1) (Khou et al., 2020) and by modulating PD-L1/PD-1 signaling (T-T Wang et al., 2017). Thus, TANs exhibit phenotype plasticity and are capable of polarizing into either an antitumorigenic “N1” phenotype or a protumorigenic “N2” phenotype (Fridlender et al., 2009). The phenotype of TANs consequently depends on the signals encountered in a given TME (Fridlender et al., 2009; Shaul and Fridlender, 2017).

PGE2 may function as a regulator of TAN polarization towards the N2 phenotype (Mizuno et al., 2019; Ohms et al., 2020). Also, PGE2/EP4 signaling in neutrophils activates PKA, which inhibits ERK to suppress neutrophil migration. Therefore, PGE2/EP4 signaling might play a role in the generation of less motile N2 neutrophils (Mizuno et al., 2014). Another study showed that EP2 facilitates neutrophil granulocyte colony-stimulating factor production (Sugimoto et al., 2005). Furthermore, PGE2 can downregulate the expression of TNF-α via EP4 and upregulate the expression of IL-6 via EP2 in neutrophils (Yamane et al., 2000).

In short, TANs display a phenotype plasticity that results in a dual role within the TME, with documented functions both in support of and against the progression of the CIC. PGE2 may play a role in regulating the polarization, the motility and the production of certain cytokines by neutrophils.

### 6.6 Mast cells
Mast cells are long-lived tissue-resident cells. They play an important role in the immune response to parasitic infections and in allergic reactions. High infiltration of tumors with mast cells is associated with poor clinical outcomes, increased vascularity, and higher tumor growth and invasion rates in many human cancers (Kashiwase et al., 2008; Groot Kormelink et al., 2009).

Mast cells have been shown to dampen antigen-specific T-cell responses through various mechanisms (Lichterman and Reddy, 2021). For one, they inhibit CD8+ T-cell function by upregulating PD-L1 expression via TNF-α (Lv et al., 2019). In a murine hepatocarcinoma model, mast cells were observed to produce chemokines that lead to the recruitment of MDSCs. These MDSCs in turn attracted Tregs in to the TME via IL-17, leading to immunosuppression (Z Yang et al., 2010). Other studies have provided evidence of the ability of mast cells to promote the recruitment of protumorigenic tumor-associated macrophages (TAMs) (Eissmann et al., 2019). Furthermore, mast cells can secrete the immunoinhibitory cytokines TGF-β and IL-10 (Wu et al., 2022).

However, antitumorigenic functions have also been ascribed to mast cells as well. As sentinel immune cells, they release multiple soluble factors that recruit other immune cells and alter their function within the TME (Lichterman and Reddy, 2021). Within the TME, they can produce chemokines that promote the recruitment and activation of T-cells, NK cells and DCs. Mast cells have also been shown to inhibit Treg functions in vitro, in a mechanism involving IL-6 (Piconese et al., 2009).

As for the links between mast cells and PGE2, it is known that PGE2 acts as a chemotactic factor for immature and mature bone marrow-derived mast cells in vitro and in vivo via the EP3 receptors (Weller et al., 2007; Kuehn et al., 2011). Additionally,
PGE2 stimulates mast cells to produce proinflammatory cytokines, chemokines, and proangiogenic factors via EP1, EP2 and EP3 (Abdel-Majid and Marshall, 2004; Nakayama et al., 2006).

In conclusion, mast cells exhibit a dual role in the tumor microenvironment, impacting both pro-tumorigenic and anti-tumorigenic processes. Their presence in tumors is associated with complex interactions involving immune cell recruitment and modulation, cytokine secretion, and angiogenesis. These diverse functions are influenced by PGE2 signaling, contributing to the complex immune landscape within the TME.

6.7 Monocytes

Monocytes function as a source for subsets of DCs and macrophages during immune responses. Human monocytes are classified into classical, nonclassical, and intermediate cells. Monocytes are therefore a heterogeneous group and can display diverse functions in the TME depending on their subtype, cancer type, signals present in the TME, stage of tumor growth and spatial positioning within the tumor (Olingy et al., 2019). Within the TME, they have the capacity to differentiate into TAMs (Schmall et al., 2015), DCs (Sheng et al., 2017) and MDSCs (Tcyganov et al., 2018). Monocyte presence in tumors has been negatively correlated with the infiltration of cytotoxic CD8+ T-cells (Lesokhin et al., 2012). In an in vivo model for hepatocellular carcinoma, it was shown that blocking monocyte infiltration into tumors leads to smaller tumors that contain less monocyte-derived TAMs and more CD8+ T-cells (Li et al., 2017). Monocyte-derived cells can also produce factors such as CCL5 that attract Tregs (Schlecker et al., 2018).
TAMs derived from classical monocytes are generally protumorigenic (Schmall et al., 2015).

There are a few reported effects of PGE2 on monocytes. *In vitro* pharmacological studies showed that EP4 signaling inhibited TNF-α production in human and murine monocytes (Meja et al., 1997; Akaogi et al., 2004). EP4 signaling also enhances the expression of the chemokine receptor CCR7 and augments the migration of monocytes (Côté et al., 2009). Furthermore, the presence of PGE2 during early development inhibits the differentiation of monocytes into functional, IL-12 producing DCs, which are essential for inducing Th1 polarization (Kaliński et al., 1997). Instead, PGE2 (via EP2 and EP4) promotes the differentiation of monocytes into DCs with a tolerogenic cytokine profile, capable of promoting the expansion of Tregs *in vitro* (Remes Lenicov et al., 2018).

In brief, monocytes represent a heterogeneous group of cells with diverse functions within the TME. Their subtypes, influenced by various factors including cancer type, TME signals, tumor stage, and spatial positioning, can differentiate into TAMs, DCs, or MDSCs. Monocytes’ presence in tumors has been associated with diverse effects that will dampen the progression of the CIC. The known effects of PGE2 on monocytes are mediated by EP2 and EP4.

### 6.8 Macrophages

For most cancers, the presence of macrophages in the tumor correlates with poor prognosis and chemotherapy resistance. However, macrophages can polarize into M1-
(proinflammatory) or M2-like (antiinflammatory) phenotypes depending on the signaling context. TAMs primarily acquire the M2-like phenotype, and their presence can be prominent within the TME (Mantovani et al., 2002; Quatromoni and Eruslanov, 2012). They stimulate angiogenesis (Lin et al., 2006) and increase tumor cell migration (Wyckoff et al., 2004), invasion (Yeo et al., 2014), and intravasation (Goswami et al., 2005; Hernandez et al., 2009). Furthermore, macrophages induce dysfunction of antitumor immune responses (Pollard, 2009; Noy and Pollard, 2014; Cassetta and Pollard, 2018). TAMs exert detrimental effects on the T-cell composition within the TME that will consequently affect CIC progression. For instance, TAMs inhibit the proliferation and function of CD8+ T-cells within the TME via IL-10 (Lepique et al., 2009) and PD-L1 expression (Jiang et al., 2017). Moreover, TAMs have been shown to recruit Tregs through chemotactic factors like CCL22 (Curiel et al., 2004).

EP2 and EP4 are the primary EPRs expressed in mouse (Ikegami et al., 2001; Akaogi et al., 2004; Pavlovic et al., 2006) and human macrophages (Bayston et al., 2003; Iwasaki et al., 2003; Kubo et al., 2004; Cipollone Francesco et al., 2005; Wu et al., 2005; Ratcliffe et al., 2007). PGE2 signaling can influence the polarization of macrophages and cause their switch from an M1 to an M2 phenotype via EP4 (Ylöstalo et al., 2012; X Wang et al., 2017; Chen et al., 2021). An in vitro study using a murine colon cancer cell line showed that overexpression of 15-PGDH has a reverse effect (Eruslanov et al., 2009). More recent studies have shown that EP4 signaling promotes M2 macrophage polarization (Barminko et al., 2014; Chang et al., 2015; Yasui et al., 2015; Zhang et al., 2015; Albu et al., 2017). However, the ratio of M1/M2 macrophages remains unchanged in the absence of EP2 (Wu et al., 2018). It should be noted that the
polarization might be context-dependent. In the context of infection, PGE2 signaling via EP4 has the opposite effect, leading to polarization toward the M1 phenotype (Sheppe et al., 2018). Signaling via the EP4 receptor has also been demonstrated to contribute to anti- and proinflammatory activity by regulating the expression of an array of cytokines and chemokines (Tang et al., 2012). The production of TNF-α (Zhong et al., 1995; Nataraj et al., 2001), IL-12 (Nataraj et al., 2001), and MCP-1 (Takayama et al., 2002) is suppressed via the EP4 receptor. The production of VEGF by M2 macrophages is potently induced via EP4 receptor signaling (W-K Wu et al., 2010; Lala et al., 2018). This signaling pathway may also be involved in regulating macrophage migration (Digiacomo et al., 2015). For the EP2 pathway, a role has been described in macrophage recruitment within the context of cardiac repair, which also involves an inflammatory microenvironment (Wu et al., 2018). EP2 activation also directly suppresses immune cell functions. It can downregulate the expression of TNF-α by macrophages (Shinomiya et al., 2001). Finally, it should be noted EP1 antagonism in an in vivo colon cancer model led to a reduced infiltration by macrophages, it did not, however, affect their polarization (O’Callaghan et al., 2013).

In summary, TAMs often contribute to angiogenesis, tumor cell migration, invasion, and intravasation while suppressing antitumor immune responses. PGE2 signaling via EP1, EP2 and EP4 influences macrophage polarization towards the M2 phenotype, the expression of various cytokines and chemokines, and modulating macrophage migration within the TME.
6.9 DCs

DCs are the most potent APCs and they play a vital role at the key steps of the CIC. Their significance is underscored by the existence of numerous ongoing clinical trials that aim to target this specific cell population within the TME (Wang et al., 2020). Not only are they exceedingly capable of priming naïve T-cells and inducing their functional polarization, but they are also responsible for the expansion and functions of T-cells in lymphoid and nonlymphoid tissues.

There are five major subtypes of DCs: plasmacytoid DCs (pDCs), type 1 conventional DCs (cDC1s), type 2 cDCs, Langerhans cells, and monocyte-derived DCs. Of these subtypes, the cDC1 group has been proposed to play a major role in antitumor immunity (Böttcher and Reis e Sousa, 2018; Böttcher et al., 2018; Cancel et al., 2019; Noubade et al., 2019). In mice, cDC1s can be further classified into CD8α+ cDCs and CD103+CD11b− cDC1s. CD8α+ cDCs can be found only in lymphoid tissues, while CD103+CD11b− cDC1s reside in nonlymphoid tissues. Upon maturation and activation, CD103+CD11b− cDC1s can migrate to the draining lymph nodes to mediate antigen presentation. They are also the only DC group that has been demonstrated to be capable of performing antigen cross-presentation. The human homologs of murine cDC1s are the (BDCA3)high CD11b−/low cDCs. Both human and mouse cDC1s express the chemokine receptor XCR1 and selectively the C-type lectin endocytic receptor CLEC9A (Vu Manh et al., 2015), two markers that separate them from other DC groups. DCs play a crucial role in the CIC, as they are directly involved in antigen-presentation,
co-stimulation and priming of de novo antigen-specific T-cells (Marciscano and Anandasabapathy, 2021).

PGE2 exerts diverse effects on DCs. In colon cancer, PGE2 was proven to suppress differentiation by inhibiting the maturation of DC from their bone marrow progenitors in vitro and in vivo (Ahmadi et al., 2008). In mice, inhibition of DC differentiation was shown to be mediated via EP2 (Yang et al., 2003). PGE2 also inhibits the ability of bone marrow-derived DCs to infiltrate tumors and perform antigen presentation by promoting endogenous IL-10 production (Harizi et al., 2002). The cause for this is the reduction of MHC-II expression and upregulation of IL-10 via EP2 and EP4 (Harizi et al., 2003).

PGE2 can regulate the effects DCs that they have on T-cell differentiation. When PGE2 is present, DCs promote T-cell tolerance by upregulating CD25 and indoleamine 2,3-dioxygenase (von Bergwelt-Baildon et al., 2006). Sheibanie et al. showed in vitro that PGE2 shifts the IL-12/IL-23 secretion axis toward IL-23 production, thereby favoring the polarization of T-cells toward the Th17 phenotype. These effects are mediated by the binding of PGE2 to the EP2 and EP4 receptors on DCs (Sheibanie et al., 2007).

The migration of DCs to tumor-draining lymph nodes is essential for T-cell priming. PGE2 regulates the migratory capacity of monocyte-derived DCs (Luft et al., 2002; Legler et al., 2006) and Langerhans cells (Kabashima et al., 2003) via EP4. CCL19/CCL21-triggered signal transduction and migration of human monocyte-derived DCs requires PGE2 to be present during their maturation (Scandella et al., 2004). Additionally, EP2 and EP4 mediate the expression of matrix metalloprotease-9 (MMP-9) in DCs. DC-derived MMP-9 is essential for DC chemotaxis in response to the CCR7 ligand CCL19 (Yen et al., 2008). PGE2 was also shown to inhibit the ability of DCs to
produce CCL19 that attracts CCR7-expressing naïve CD4+ T-cells (Muthuswamy et al., 2010). In mice, only EP4 regulates the migration of DC, although both EP2 and EP4 are expressed in these cells (Kabashima et al., 2003).

In a nutshell, DCs hold a central and indispensable role in the CIC, where they are directly involved in antigen presentation, co-stimulation, and the priming of antigen-specific T-cells. Within the TME, PGE2 exerts diverse effects on DCs, influencing their differentiation, migration, and modulation of T-cell responses. Understanding the intricate relationship between PGE2 and DCs is essential for unraveling some of the complexities of cancer immunology and may have significant implications for the development of novel immunotherapies.

6.10 Myeloid-derived suppressor cells (MDSCs)

MDSCs are a heterogeneous subset of immune cells. The group consists mainly of immature forms of myeloid cells. They have been described as having immunosuppressive functions in cancer. Thus, ROS, PGE2, PD-L1 expression and STAT-3 signaling stemming from MDSC have been shown to suppress the ability of CD8+ T-cells to mediate effective responses (Yamaoka et al., 2009; Poschke et al., 2010; Li et al., 2021). Furthermore, MDSCs suppress natural killer (NK) cell responses in a TGF-β-dependent manner (Mao et al., 2014). Obermajer et al. showed that PGE2 and COX2 redirect the differentiation of human DCs toward stable MDSCs via a positive feedback mechanism. They also showed that the pharmacological treatment of MDSCs with EP2 and EP4 antagonists reverses the suppressive functions of MDSCs (Obermajer et al., 2011). In a different study, the coculture of monocytes enriched from healthy individuals with melanoma cell lines induced the monocytes to acquire an
MDSC-like phenotype and suppressive properties mediated by COX2/PGE2 and STAT-3 signaling (Mao et al., 2013). Overexpression of COX2 in tumor cells was also shown to lead to MDSC accumulation and reduce NK cell cytotoxicity in vivo (Mao et al., 2014). In conclusion, MDSCs are a heterogeneous subset of immune cells that are associated with immunosuppressive functions in cancer, by suppressing key elements of the CIC, such as CD8⁺ T-cells and NK cells. Antagonists targeting EP2 and EP4 receptors have shown promise in reversing the suppressive functions of MDSCs.

6.11 NK cells

NK cells are a group of innate effector lymphocytes that work at the interface between innate and adaptive immunity (Moretta et al., 2008). They are involved in the response to a wide array of pathological stimuli. NK cell functions range from killing virally infected cells to the early detection and control of tumorigenic development via the lysis of cancerogenic cells. They can recognize and lyse tumor and virus-infected cells without previous sensitization (Moretta et al., 2002). Specifically, NK cells produce perforin and granzyme B to penetrate target cells and kill them (Caligiuri, 2008). NK cell subsets display major functional differences in their cytolytic activity and cytokine production (Cooper et al., 2001). Apart from their cytotoxic activity, these immune cells can engage in reciprocal interactions with DCs (Carbone et al., 1999; Fernandez et al., 1999; Wilson et al., 1999; Ferlazzo et al., 2002; Gerosa et al., 2002; Moretta, 2002; Piccioli et al., 2002; Cooper et al., 2004; Walzer et al., 2005), macrophages (Long, 2007; Nedvetzki et al., 2007), T-cells (Lu et al., 2007), mast cells (Portales-Cervantes et al., 2019), eosinophils (Awad et al., 2014; Pesce et al., 2017), neutrophils (Costantini et al., 2010,
2011; Costantini and Cassatella, 2011; Riise et al., 2015), and endothelial cells (Berman et al., 1996; Dondero et al., 2017). These crosstalk interactions can either limit or exacerbate the immune responses (Zitvogel, 2002).

NK cells can exert influence on the progression of the CIC through several mechanisms. Firstly, they contribute to the cycle by directly eliminating tumor cells using various cytotoxic mechanisms, leading to the release of tumor antigens into the TME (Chu et al., 2022). These mechanisms include inducing apoptosis in tumor cells through the actions of perforin and granzyme B, initiating apoptosis via NK surface molecules like FasL and TRAIL (Prager et al., 2019), and participating in antibody-dependent cell-mediated cytotoxicity (ADCC) (Li and Liu, 2022). The second mechanism that NKs can utilize to influence the CIC, is by producing cytokines that lead to the recruitment and activation of adaptive immune cells. For instance, their secretion of IFN-γ can activate CD8^+ T-cells (Alspach et al., 2019) and promote the polarization of Th1 cells (Martín-Fontecha et al., 2004), enhancing the adaptive immune response. Lastly, NK cells can also contribute to the antigen presentation step of the CIC. They can activate and deliver antigens to DCs, prompting DCs to engage in antigen cross-presentation with CD8^+ T-cells (Peterson and Barry, 2020). It's worth noting that recent research has unveiled some plasticity in the behavior of NK cells within the TME (Corvino et al., 2022). Unique subsets of NK cells that exist specifically in the TME have demonstrated reduced cytolytic functions (Gonzalez et al., 2021) and the capacity to promote angiogenesis (Bruno et al., 2013). However, further investigation is required to fully comprehend the characteristics and functional outcomes of these distinct NK cell subsets in the context of cancer immunology.
Human NK cells express all EPRs, except for EP1 (Martinet et al., 2010), activated NK cells only express EP2 and EP4 at the mRNA level (Walker and Rotondo, 2004). PGE2 in the TME inhibits NK cells, which has been demonstrated in multiple studies, both in vivo and in vitro (Jin et al., 2023). Studies also showed that the inhibitory effects exerted by PGE2 on NK cells are mainly mediated by EP4 (Holt et al., 2011; Ma et al., 2013) and EP2 (Walker and Rotondo, 2004). In tumor-bearing mice, PGE2 inhibits NK cell migration, cytotoxicity, and differentiation via EP2 and EP4 (Martinet et al., 2010; Park et al., 2018). It has been shown that the inhibition of cytotoxicity involves suppression of IL-12 and IL-15 responsiveness (Joshi et al., 2001; Walker and Rotondo, 2004). Moreover, PGE2 inhibits proliferation and induces apoptosis of NK cells via EP2 and EP4 (Li et al., 2016). PGE2 binding of EP2 and EP4 also downregulates NK cell receptor expression via a cAMP/PKA pathway (Martinet et al., 2010). Furthermore, PGE2 inhibits the ability of NK cells to secrete IFN-γ via EP2 in cAMP dependent manner (Park et al., 2018), attenuating IL-12- or IL-18-induced IFN-γ expression (Walker and Rotondo, 2004). IFN-γ secreted by NK cells stimulates other immune cell types, thereby activating immune responses. The function of NK cells in the DC-mediated induction of Th1 and cytotoxic T-lymphocyte responses is abolished by inhibiting IFN-γ (Mailliard et al., 2005; Caligiuri, 2008). PGE2 has also been demonstrated to have a negative effect on the crosstalk between NK cells and DCs. The known effects that PGE2 has on the reciprocal crosstalk between DCs and NK cells (specially via EP2 and EP4) and the effects on T-cell polarization were well summarized in the review by Hedi Harizi (Harizi, 2013). The PGE2 effects in this NK-DC-T-cell axis could lead to immune escape and will be further discussed later in this review.
In summary, NK cells play a multifaceted role at the interface of innate and adaptive immunity, with significant contributions to immune responses against various pathological stimuli, including cancer. Their diverse functions can influence various stages of the CIC. PGE2 in the TME has been found to inhibit NK cells through the EP2 and EP4 receptors, impacting their migration, cytotoxicity, differentiation, proliferation, and cytokine secretion. This inhibition can negatively affect the crosstalk between NK cells and other immune cells like DCs and contribute to immune escape within the TME.

7 PGE2 and the CIC: possible mechanisms of immune evasion

Taken together, these data suggest that the effects that PGE2 has on immune cells can lead to the disruption of the CIC at several key steps (Figure 1 and Table 2). The binding of PGE2 to EPRs expressed on T-cells initiates a cascade of immunoinhibitory signals that block T-cell proliferation and IL-2 production (Paliogianni et al., 1993). PGE2 can also inhibit T-cell functions indirectly by inducing the synthesis of immunosuppressive cytokines such as IL-10. Additionally, the presence of PGE2 not only leads to lower activation (by promoting PD-1 expression) and proliferation levels of CD8+ and CD4+ T-cells (Gorchs et al., 2019) but also promotes cytotoxic T-cell exhaustion (Xun et al., 2021).

PGE2 also has regulatory functions on elements of the innate immune system shown to be key in early antitumor responses. Such is the case with conventional NK (cNK) cells. PGE2 negatively affects the cytotoxic capacity of cNK cells in vitro, as shown in
coculture experiments using conditioned media of thyroid cancer cells (Park et al., 2018). However, the relevance of cNK cells in antitumor immunity is not limited to their cytotoxic capacities but involves their ability to interact and recruit other cellular elements of the immune system to the tumor site via chemokine production. Elevated levels of PGE2 in the TME have proven to reduce the chemokine production of cNK cells and their viability, thereby limiting their antitumor effects within the TME. A prominent effect of PGE2 on immune cells is the suppression of myeloid cell activation and recruitment into the TME (Zelenay et al., 2015). By inhibiting cNK cell functions, PGE2 leads to a lower presence of cDC1 in the TME. This is because, besides their cytotoxic functions, NK cells have been demonstrated to be key factors in the recruitment and differentiation of cDC1s by producing chemokines such as XCL1 and CCL5 (Böttcher et al., 2018) and formative cytokines for cDC1s and FLT3L (Barry et al., 2018).

cDC1s are a group of DCs that, albeit few, fulfill several functions that are critical for driving immune responses to cancer. Besides performing antigen presentation via the MHC-II, they can also perform antigen cross-presentation via the MHC-I (Broz et al., 2014), being the only antigen-presenting DC type that can do this. Therefore, the absence of functional cDC1s negatively affects antigen priming of CD8$^+$ T-cells. Not only the activation but also the translocation of effector CD8$^+$ T-cells to the tumor from tumor-draining lymph nodes is strongly driven by cDC1. They produce the T-cell chemoattractant CXCL9 and CXCL10 at high levels (Spranger et al., 2017; de Mingo Pulido et al., 2018). The sequential failure to recruit cDC1s and CD8$^+$ T-cells into the tumor provoked by the presence of PGE2 results in poorly infiltrated tumors and may
lead to ICI therapy resistance. In human melanoma, the numbers of NK cells and cDC1s correlate with increased OS and patient responsiveness to anti-PD-1 immunotherapy (Salmon et al., 2016; Barry et al., 2018). Bonavita et al. provided further evidence for this immune axis. They showed that NK cells drive inflammatory remodeling of T-cell-inflamed tumors. Furthermore, the actions of PGE2 on EP2 and EP4 receptors of NK cells can act as a switch that enables immune escape (Bonavita et al., 2020).

Later, Thumkeo et al. showed that in a LLC1 mouse model, PGE2 leads to immunosuppression in the inflammatory TME. These effects are mainly mediated by EP2 and EP4 receptors expressed on tumor-infiltrating myeloid cells, specific DC populations and Tregs. They include inflammatory myeloid cells mediating angiogenesis, DCs producing chemokines that promote Treg infiltration (Ccl22 and Ccl17) and direct Treg activation by PGE2 mediated signaling. Pharmacological antagonism of the EP2 and EP4 receptors in this model lead to a decrease in tumor size. ScRNAseq analysis showed that the treatment caused significant changes in the expression of genes related to angiogenesis (myeloid cell populations), Ccl22 and 17 production (DCs) and Treg activation. Furthermore, treatment with EP2/EP4 antagonists lead to significant decrease of TANs (Thumkeo et al., 2022). In a recent publication, Bayerl et al. identified the mechanism by which PGE2 showed in an in vivo model that, even when cDC1 cells are present in the TME, PGE2 reprograms these cells into a dysfunctional state via the EP2 and EP4 receptors (Bayerl et al., 2023).
8 PGE2, EPR antagonists, and immunotherapy

A wide array of EPR antagonists have been discovered. However, antagonists for a single EPR can differ from each other greatly in terms of selectivity, potency and species affinity (Lebender et al., 2018). An overview of the known antagonists for each EPR can be found in Table 1. The EP4 receptor is considered a potential novel therapeutic target in cancer due to its prominent role in the human TME. EP4 antagonists could be clinically used as adjuvant therapies along with other immune treatments to increase their efficacy. There is mounting preclinical evidence in favor of this proposed strategy. A previous study showed that EP4 antagonism synergizes with either Treg depletion or an anti-cytotoxic T-lymphocyte antigen 4 (anti-CTLA-4) antibody treatment (Albu et al., 2017). In line with the immune axis presented in this review, EP4 antagonist therapy blocks the PGE2-mediated dysfunction of NK cells (Ma et al., 2013), which, in turn, can sequentially reestablish the infiltration of cDCs and CD8$^+$ T-cells into the TME, thereby reestablishing antitumor immunity. Further publications have shown that EP4 antagonism synergizes with anti-PD1 treatment in vivo. The observed effects were linked to the downregulation of tumor metabolism and the modulation of lymphocytes and myeloid cells. In these studies, EP4 antagonism was shown to affect immune cell infiltration in tumors and impact the function of specific immune cell subsets, such as MDSCs, NK cells, and CD8$^+$ T-cells (Wang et al., 2021; Tokumasu et al., 2022). At the time of this review, a phase-1 clinical trial with the EP2 and EP4 antagonist TPST-1495 as a single agent and with pembrolizumab is recruiting patients with advanced solid tumors according to clinicaltrials.gov, (ClinicalTrials.gov identifier:
NCT04344795). Another study on advanced NSCLC, designed to assess the EP4 antagonist grapiprant along with pembrolizumab, was terminated in February 2021 (ClinicalTrials.gov Identifier: NCT03696212). Further clinical trials involving grapiprant in the context of different cancer entities have either recently been completed or are currently in progress (e.g. NCT05041101 for breast cancer and NCT03658772 for colorectal cancer). Results for a phase I clinical study in patients with advanced cancers using EP4 antagonist E7046 were published by Hong et. al in 2020 (ClinicalTrials.gov Identifier: NCT02540291) (Hong et al., 2020). Further information about clinical trials and future directions for EP4 antagonists in cancer can be found in the reviews by Ching et al. (Ching et al., 2020), Majmuder et al. (Majumder et al., 2018) and Mizuno et al. (Mizuno et al., 2019). Current known antagonist for EPRs, included those only used for research purposes, are summarized in Table 1. The list of antagonists and clinical trials presented in this review is meant to be comprehensive, however, it may be incomplete.

In conclusion, EP receptor antagonism, particularly of EP4 and EP2 holds promise as a novel therapeutic strategy in cancer. The effects these antagonists have on immune cells of the TME give them potential as adjuvant therapies to immune treatments. Preclinical evidence suggests that EP4 antagonism can synergize with various immune-based strategies, such as Treg depletion and immune checkpoint inhibition. Ultimately, these synergistic effects can promote the reestablishment of antitumor immunity in patients. Human clinical trials are underway to evaluate the efficacy and safety of EP4 and double EP2/EP4 antagonists, both as single agents and in combination with other immunotherapies. These trials represent a promising avenue for the development of new cancer treatments.
Acknowledgments

We would like to thank all members of the Kargl lab for fruitful discussions.

Data Availability Statement

This review article contains no datasets generated or analyzed during the current study.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Santiso A, Heinemann A, Kargl J
Figure legends

Figure 1. The cancer-immunity cycle (CIC) and possible disruptions caused by PGE2. Prostaglandin E2 (PGE2) effects can lead to disruption of the cycle at several key steps. The prostaglandin affects the recruitment and functionality of immune cell populations that take part in antigen presentation (steps 2 and 3 of the CIC). PGE2 can also indirectly inhibit the trafficking and infiltration of cytotoxic T lymphocytes to the TME (steps 4 and 5) by modulating chemokine production and by promoting immune cell phenotypes like immunosuppressive myeloid cells that are disruptive to CIC. Within the TME, binding of PGE2 to EP receptors (EPRs) expressed on T-cells can hinder their activation, inhibit their proliferation, and provoke their exhaustion. Thereby interfering with steps 6 and 7 of the CIC. Figure created with BioRender.com

Main PGE2 metabolism pathways. Arachidonic acid (AA) is cleaved from the cell membrane by cytosolic phospholipase A2 (cPLA2). Cyclooxygenase (COX) enzymes convert AA to prostaglandin H2 (PGH2). PGH2 is then converted to PGE2 via the terminal synthases mPGES1/2 or cPGES. The biological inactivation of PGE2 is catalyzed by 15-PGDH. Figure created with BioRender.com
Table 1. EP receptor subtypes, their main signaling pathways, affinity for PGE2, and published antagonists

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<th>Subtype</th>
<th>Isoforms</th>
<th>Coupled G-proteins</th>
<th>Signaling pathways</th>
<th>$K_i$ values for PGE2 [nM]</th>
<th>Desensitization</th>
<th>Antagonists</th>
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- ONO-AE3-208 (Murase et al., 2008; Gill et al., 2016)
- E7046 (Albu et al., 2017) (Hong et al., 2017) (Hong et al., 2020) (Pi et al., 2022)
- ASP7657 (Mizukami et al., 2018) (Thumkeo et al., 2022)
- AAT-008 (Manabe et al., 2023)
- Grapiprant (Sartini and Giorgi, 2021)
- GW627368X (Wilson et al., 2018)
Table 2. Immune cell types that respond to PGE2 signaling, relevant receptors, outcome and species.

<table>
<thead>
<tr>
<th>Immune cell type</th>
<th>PGE2 receptor</th>
<th>Effects that influence initiation/progress of the CIC (species)</th>
</tr>
</thead>
</table>
| CD8+ T-cell      | EP1<sup>2</sup>, EP2<sup>1,2</sup>, EP4<sup>1,2</sup> | PD-1 expression<sup>1</sup>  
upregulation of the expression of inhibitory receptors<sup>1</sup>  
Attenuated IFN-γ release<sup>2</sup>  
inhibition of proliferation<sup>1,2</sup>  
promotion of replicative senescence<sup>1</sup>  
activation of immunosuppressive cell types<sup>1,2</sup> |
| CD4+ T-cells     | EP1<sup>2</sup>, EP2<sup>1,2</sup>, EP4<sup>1,2</sup> | Suppression of Th1 activation<sup>1</sup>  
Suppression of Th1 proliferation<sup>1</sup>  
Reduced Th1 cytokine production<sup>1</sup>  
Induction of Th1 differentiation<sup>2</sup>  
Polarization towards Th2 phenotype<sup>1</sup>  
Th17 expansion<sup>1,2</sup>  
Induced expression of Foxp3<sup>2</sup>  
Treg infiltration<sup>2</sup> |
| B-cells          | EP2<sup>1</sup>, EP4<sup>1,2</sup> | Inhibition of activation<sup>1,2</sup>  
Repression of MHCII expression<sup>2</sup> |
<table>
<thead>
<tr>
<th>Cell Type</th>
<th>EP Subtypes</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils</td>
<td>EP2, EP4</td>
<td>Promotion of IgE production, suppression of chemotaxis, degranulation, various functions (ROS production, etc.)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>EP2, EP4</td>
<td>Inhibition of migration, Polarization towards N2 phenotype, production of granulocyte colony-stimulating factor, downregulation of expression of TNF-α, upregulation of the expression of IL-6</td>
</tr>
<tr>
<td>Mast cells</td>
<td>EP1, EP2, EP3</td>
<td>Chemotaxis, production of proinflammatory cytokines, chemokines and proangiogenic factors</td>
</tr>
<tr>
<td>Monocytes</td>
<td>EP2, EP4</td>
<td>TNF-α production, augmented migration, inhibition of development into certain DC phenotypes</td>
</tr>
<tr>
<td>Macrophages</td>
<td>EP1, EP2, EP4</td>
<td>Recruitment, polarization towards M2 phenotype, expression of cytokines and chemokines, infiltration</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>EP2, EP4</td>
<td>Inhibition of differentiation</td>
</tr>
<tr>
<td>Cell Type</td>
<td>EP2, EP4</td>
<td>Functions</td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
<td>-----------</td>
</tr>
</tbody>
</table>
| MDSCs     | EP2, EP4| inhibition of antigen presentation\(^2\)  
promotion of IL-23 production\(^2\)  
regulation of migration of monocyte derived DCs\(^1\)  
maturation of monocyte derived DCs\(^1\)  
migration\(^1\)  
MDSC differentiation\(^1\)  
suppressive functions\(^1\) |
| NK cells  | EP2, EP4, EP4\(^1,2\) | inhibition of migration\(^2\)  
inhibition of differentiation\(^1,2\)  
inhibition of proliferation\(^1,2\)  
inhibition of cytotoxicity\(^1,2\)  
induction of apoptosis\(^1\)  
downregulation of NK cell receptor expression\(^1\)  
inhibition of IFN-\(\gamma\) expression\(^2\) |
11 Footnotes

This work was funded by the Austrian Science Fund: [FWF-P35294] and the FWF doctoral program: [DK-MOLIN W1241], Open Access Funding by the Austrian Science Fund (FWF).

No author has an actual or perceived conflict of interest with the contents of this article.
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induced gene expression of cytokines in human macrophages. *Immunology* **84**:446–452.


Fig. 1

1. **Release of cell antigens**
   - PD1 expression ↑
   - IFN-γ release ↓
   - Inhibitory receptors ↑
   - Replicative senescence ↑

2. **Cancer antigen presentation**
   - DC
   - Differentiation ↓
   - Tumor infiltration ↓
   - Antigen presentation ↓
   - T-cell tolerance ↑

3. **Priming and activation of APCs and T cells**
   - B-cell
   - Proliferation ↓
   - Differentiation ↓
   - Activation ↓
   - MHC expression ↓
   - IgE production ↑

4. ** Trafficking of CTLs to tumor**
   - NK cell
   - Proliferation ↓
   - Apoptosis ↑
   - NK-DC crosstalk ↓
   - CD4+ T-cell
   - Activation ↓
   - Proliferation ↓
   - Th2 polarization ↑

5. **Infiltration of T-cells into the tumor**
   - MDSC
   - Activity ↑
   - Differentiation ↓
   - Suppressive functions ↑
   - Presence in the TME ↑

6. **Recognition of cancer cells by CTLs**
   - Macrophage
   - M2 activation ↑
   - Regulation of cytokine expression
   - Tumor infiltration ↓

7. **Killing of cancer cells**
   - Treg activity ↑
   - Expression of Foxp3 ↑
   - Infiltration ↓

- **Tumor cell**
- **Apoptotic tumor**
- **EP receptor**
- **PGE2 effects**