The complex role of Prostaglandin E_2-EP receptor signaling in wound healing.

Kristy E. Gilman[1*] and Kirsten H. Limesand[1]

[1] The University of Arizona, Department of Nutritional Sciences, Tucson, AZ 85721, USA
*Corresponding Author
Kristy E. Gilman
1177 E 4th St.
Shantz 438
Tucson, AZ 85721

Email: gilmankr@email.arizona.edu

Key words:
Prostaglandins, tissue damage, wound healing, cell signaling, repair

Abstract

Prostaglandins are critical lipid mediators involved in the wound healing response, with prostaglandin E_2 (PGE_2) being the most complex and exhibiting the most diverse physiological outputs. PGE_2 signals via four G-protein coupled receptors, termed EP-receptors 1-4, that induce distinct signaling pathways upon activation and lead to an array of different outputs. Recent studies examining the role of PGE_2 and EP receptor signaling in wound healing following various forms of tissue damage are discussed in this review.

Introduction

Wound healing is a highly complex and coordinated process that has three sequential but overlapping phases: (i) hemostasis/inflammation- consisting of vascular changes, immune infiltration, and inflammatory signaling; (ii) proliferation- focusing on reepithelialization and angiogenesis; and (iii) remodeling- aiding in maturation and strengthening of the newly generated tissue (7). Following a wounding event, these processes must be tightly regulated to ensure that proper healing occurs. Dysregulation of any stage of the response can lead to either insufficient repair or chronic inflammation and excessive tissue damage. Therefore, coordinated inflammation is critical for a wound to heal properly, which requires a number of different cell types and inflammatory signals. Here, we focus on the role of prostaglandin E_2 (PGE_2) in the response to tissue injury and the important roles of this signaling mediator to induce proper wound healing. Additionally, we discuss recent discoveries related to PGE_2 and wound repair.

Prostaglandin E_2 (PGE_2) synthesis and signaling

Prostaglandins (PGs) are a family of bioactive lipids that exert a wide range of physiological effects throughout the body and are commonly involved in vasodilation, tissue repair/homeostasis, and inflammation. PGs are enzymatically synthesized from plasma membrane-derived phospholipids. These phospholipids are cleaved by the action of

phospholipase A_2 (PLA_2), which releases fatty acids into the cytosol to be acted upon by a variety of enzymes (21). Arachidonic acid (AA) is the predominant fatty acid that is released, which is converted to prostaglandin G_2 (PGG_2) and PGH_2 via a two-step reaction by cyclooxygenase (COX) enzymes, including the constitutively active isoform COX-1 and the inflammation-inducible isoform, COX-2. PGH_2 is then subsequently acted upon by PGE synthase enzymes to generate PGE_2. While some prostaglandins are preferentially synthesized from COX-1 specific or COX-2 specific products, PGE_2 can be generated from both COX-1 and COX-2 derived PGH_2 (22). PGH_2 is acted on by one of three isoforms of PGE synthase enzymes: microsomal PGE synthase-1 (mPGES-1), mPGES-2, and cytosolic PGE synthase (cPGES). Generally, mPGES-1 is considered the inflammation-activated isoform, while mPGES-2 and cPGES are both constitutively expressed in most cell types (19). PGE_2 is secreted by most cells throughout the body and is inactivated by the enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH), which oxidizes PGE_2 to form 15-keto-PGE_2 (30).

PGE_2 exerts varying effects depending on the concentration of PGE_2 present, the cell/tissue type and the receptor isoform (EP 1-4) of which it interacts with (21). For these reasons, PGE_2-EP receptor signaling is highly complex yet crucially important for an appropriate inflammatory response (Figure 1). EP receptors are PGE_2-specific G-protein coupled receptors (GPCRs) that are widely expressed throughout the body and induce distinct intracellular signaling cascades via activation of their respective G proteins: EP1 utilizes G_q, EP3 utilizes G_i and EP2 and EP4 utilize G_s (26). These G-proteins activate different downstream signaling pathways that lead to either pro- or anti-inflammatory responses (17) . EP1 activates phospholipase C (PLC), leading to increased diacylglycerol (DAG) and inositol triphosphate (IP3), increasing intracellular calcium levels, and subsequent protein kinase C (PKC) activation (21). EP2 and EP4 receptors both activate two identical signaling cascades: the first entails activation of adenylate cyclase

causing increased cyclic adenosine monophosphate (cAMP) production and subsequent protein kinase A (PKA) and cAMP response element binding protein (CREB) activity; the second includes activation of phosphoinositol-3-kinase (PI3K) and Akt via the β-arrestin pathway following EGFR transactivation (1). EP3 receptor activation leads to inhibition of adenylate cyclase and reduced cAMP levels, contradicting EP2/4 signaling. In general, EP1 and EP3-induced signaling are considered anti-inflammatory while EP2 and EP4 are considered pro-inflammatory (21). EP receptor distribution varies across tissues with EP3 and EP4 being the most commonly expressed subtypes found almost ubiquitously throughout cell types and EP1 and EP2 being present in only select tissues (25). Tissue mRNA and protein content of EP receptors can be evaluated via the Human Protein Atlas (26) and a more detailed description of arachidonic acid metabolism and prostaglandin signaling can be found in previously published reviews (4, 19, 21).

PGE_2 is produced following tissue injury

PGE_2 is the most complex prostaglandin due to the multiple receptor isoforms that each induce distinct signaling cascades and outputs. The opposing signaling pathways initiated by PGE_2 make it difficult to determine whether PGE_2 is beneficial or detrimental to the healing response. Many types of tissue injury are described below, with the commonality of each of the studies described being that PGE_2 is produced following injury. For example, in DSS-induced colitis, PGE_2 levels were elevated during the induction phase, at day-4 post DSS initiation, that coincided with increased PLA_2 and COX-2 expression, which was sustained for two weeks (8). PGE_2 levels were also increased following dermal excisional wounding, with the highest concentration observed days 1-3 following injury with levels remaining elevated above baseline through day 12 (5). While these studies suggest PGE_2 is important for the wound healing process, more details describing how PGE_2 exerts its effects are necessary to fully understand the potentially beneficial and/or detrimental pathways induced by PGE_2-EP signaling.

PGE_2 modulates cell physiology

EP1 and EP3-signaling have been shown to induce vasoconstriction, while EP2 and EP4-signaling lead to vasodilation (10). In general, vascular alterations are necessary for tissue repair and these observations alone are not indicative of promoting or impeding wound healing. In the murine renal vasculature system, EP3 activation by PGE_2 led to constriction of the vasculature and EP3-deficient mice showed a reduced ability to vasoconstrict (13). Interestingly, varying levels of PGE_2 have been shown to regulate cerebral blood flow in a dichotomous fashion; low-level-PGE_2 induced EP4 activation led to vasodilation, while high-level-PGE_2 induced EP1 activation led to vasoconstriction in cerebral parenchymal arterioles, with both phenotypes inhibited by antagonism of EP4 and EP1, respectively (3). Further, in vascular smooth muscle cells, deletion or inhibition of EP4 led to increased vascular constriction and increased blood pressure following stimulation with Angiotensin-II, while EP4 agonism inhibited this phenotype (29). PGE_2-EPR regulation of vascular flux is therefore necessary for the beginning stages of wound healing to prevent blood loss upon immediate injury and to allow for increased blood flow and immune infiltration at later time points. Pharmacological modulation of PGE_2 concentration could provide a critical therapeutic benefit for individuals needing alterations in vascular pressure following injury.

PGE_2 is also able to induce differentiation of cell subtypes. Tendon-derived stem cells isolated from rats that were treated with PGE_2 display osteogenic differentiation by increasing protein content of bone-specific proteins, bone morphogenic protein-2 (BMP2), runt-related transcription factor 2 (Runx2), osterix (OSX) and osteocalcin (OCN) along with increased alkaline phosphatase activity and calcium deposition (34). Additionally, in zebrafish intestinal tissue and human-derived intestinal organoids, PGE_2 treatment increased mucus production, indicative of intestinal differentiation toward a goblet cell phenotype (2). PGE_2 can also influence

macrophage polarization and metabolism. In bone marrow derived macrophages (BMDM), IL-4 and PGE_2 cotreatment induced M2 macrophage polarization, an anti-inflammatory macrophage phenotype that is represented an increase by M2 markers, Arginase (*Arg1*), Mannose receptor C-type 1 (*Mrc1*), Resistin-like-α (*Fizz1)* and Chitinase type protein 3 (*Ym1*) due to CREB-Krupple-like factor 4 (KLF4) mediated transcription (15). Additionally, PGE_2 treatment following IL-4-induced M2 macrophage polarization modulated metabolic pathways by reducing mitochondrial membrane potential and subsequent ATP production due to reduced transcription of malate-aspartate shuttle components that led to an overall decrease in intracellular reactive oxygen species (23). Further, in mice with ear hole punch injuries, EP3 and EP4 were shown to influence M2 macrophage recruitment and polarization, with EP3 and EP4 deficient mice exhibiting altered ratios of M1 to M2 macrophages (11). These studies illustrate how PGE_2 treatment can alter specific cell physiology in a manner that could be either beneficial or detrimental to an injured site, depending on the context.

PGE_2 in promoting wound healing

EP-receptor mediated phenotypes

PGE_2-EP receptor signaling positively influences wound healing by altering immune migration, increasing proliferation and promoting survival. In a rodent model of myocardial infarction (MI), EP2 activation in macrophages was critical for macrophage recruitment to the injured site with EP2 deficient mice exhibiting increased infarct size, reduced immune cell mobilization and poorer tissue regeneration than wildtype counterparts (27). Additionally, mice deficient in EP3 or EP4 exhibit reduced wound closure following ear hole punch injury that correlated with reduced macrophage recruitment to the injured site, an overall reduction in M2 macrophages and a reduction in new lymph and blood vessel formation that coincided with reduced mRNA of vascular endothelial growth factor receptor-3 (VEGFR3) and lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) in granulation tissue compared to wildtype mice (11).

Corroborating these results, mice receiving bone marrow transplants exhibited increased hematopoiesis, chemokine production, colony formation and immune cell homing post-transplant that correlated with increased survival, which was greatly reduced with either EP2 or EP4 antagonism (32). Interestingly, inhibiting EP4 signaling has also been shown to increase immune infiltration at sites of injury. Mice lacking EP4 on vascular smooth muscle cells (VSMCs) present with increased immune infiltration, aortic size, and oxidative stress following angiotensin-II (Ang-II)-induced damage to the vasculature (29). Similarly, in angioplasty-wire injured mice, EP4 specific deletion on endothelial cells increased immune infiltration and impeded tissue repair, whereas EP4 agonism improved endothelial repair and reduced neointima formation in wildtype mice (9). Likewise, zebrafish with amputated tail fins required EP4 signaling to eliminate neutrophils from the injured site to allow for continued tissue repair post-injury (14). These studies suggest a dual role for EP receptor signaling, especially EP4, in mediating immune infiltration and egress during tissue repair.

EP2 and EP4 also induce proliferation post-injury to aid in tissue regeneration. EP4 signaling following angioplasty wire injury was shown to be important for reendothelialization, due to EP4-cAMP-PKA induced proliferation, that occurred to a lesser extent following EP2 activation (9). In mice with DSS-induced colitis, PGE_2 treatment increased expression of EP4 that led to β-arrestin-PI3K-Akt signaling which increased proliferation and reduced disease severity and apoptosis levels (20). Interestingly, samples from human colitis patients or DSS-treated mice show a reduction in EP4, COX-1 and PGE_2 content but increased COX-2 levels compared to healthy controls (20). Together, these studies show the importance of EP4, and to a lesser extent EP2 signaling, in proliferation induction to allow for reepithelialization at the injured site to enhance tissue repair.

Modulation of PGE_2 concentration

Modifying PGE_2 levels via treatment with PGE_2, PGE analogs or by impeding PGE_2 inactivation can improve wound healing phenotypes. Supporting this, mice deficient in the PGE_2 inactivating enzyme, 15-PGDH, have elevated PGE_2 levels in bone marrow and liver and have improved regenerative abilities and reduced markers of tissue damage following bone-marrow transplantation, or hepatectomy-induced liver regeneration, respectively, that was also observed following pharmacological 15-PGDH blockade (32). In a mouse model of DSS-induced colitis, PGE_2 treatment reduced symptom severity and apoptosis levels while increasing colonic repair and proliferation via upregulation of EP4-dependent β-arrestin-PI3K-Akt signaling (20). Corroborating these results, genetic or pharmacological inhibition of 15-PGDH led to increased colonic PGE_2 content that correlated with reduced histological markers of colitis and increased proliferation of intestinal tissue 7 days following DSS treatment in mice (32). Further, in a zebrafish model of DSS-induced intestinal injury, PGE_2 treatment increased mucus production, barrier integrity, autophagy and cell viability, reduced *E.coli* uptake, and improved overall survival following injury (2). Interestingly, treatment of cutaneous wounds with PGE_2-incorporated hydrogels improved speed of recovery by enhancing angiogenesis, reducing inflammation and immune infiltration and shortening the time of wound closure (31). Additionally, in mice with bleomycin-induced pulmonary fibrosis, 15-PGDH inhibition reduced markers of fibrosis, inflammatory cytokine expression in lung and serum, and improved airway function that correlated with increased survival (24). Lastly, following angioplasty wire injury, treatment of mice with a PGE analog, misoprostol, increased reendothelialization and improved healing, whereas loss of mPGES-1 blocked tissue repair (9). Combined, these studies illustrate the essential role of PGE_2-induced reparative tissue responses following injury and suggest that increasing PGE_2 concentration at sites of damage may improve wound resolution.

PGE_2 treatment can also alter macrophage polarization and metabolism, leading to an M2, anti-inflammatory macrophage phenotype (23), which has been observed in white adipose tissue in

an obesity model (15), in skin following cutaneous wounding (27) and in microglia following traumatic brain injury (28). As mentioned previously, macrophages treated with PGE_2 present markers of M2 macrophages, including *Arg1, Mrc1, Fizz1* and *Ym1* that is dependent on CREB-KLF4 induced transcription (15) and can modify mitochondrial membrane potential and transcription of malate-aspartate shuttle components to reduce ATP production (23). Interestingly, macrophage proliferation is influenced by PGE_2 signaling in a dose-dependent manner, with the highest rate observed following 1μM PGE_2 treatment *in vitro,* that leads to M2 macrophage polarization and increased expression of Arginase, interleukin (IL)-1 receptor a, IL-10, CD68 and CD206 with reduced M1 markers, IL-1β, IL-6 and TNFα (31). Mice with skin wounds that are treated with PGE_2 incorporated hydrogels have increased M2 macrophages present at the site of injury that correlated with increased angiogenesis, faster wound closure, and reduced scarring (31). These signaling pathways are critical during a healing response, as the transition from a pro-inflammatory to an anti-inflammatory immune cell phenotype will directly dictate a tissue's ability to begin the remodeling phase of the response.

Inhibiting PGE_2 production with COX inhibitors can be detrimental to wound healing. Following mouse ear hole punch injury, treatment with the selective COX-2 inhibitor, celecoxib, reduced wound closure due to decreased macrophage recruitment and M2 macrophage numbers, and reduced blood and lymph vessel formation at the injured site due to decreased transcriptional content of LYVE-1, VEGFR3, VEGF-C and VEGF-D (11). In a rodent model of bone injury, treatment with the non-steroidal anti-inflammatory drug, naproxen, led to a reduction in serum PGE_2 levels which correlated with poorer woven bone formation following mechanical loading, indicating a need for PGE_2 signaling in bone remodeling post-injury (18). Together, these studies underline the importance of PGE_2-mediated signaling following injury, but more studies defining the role of EP receptors in these responses would be highly beneficial.

PGE_2 in impaired wound healing

EP-receptor mediated phenotypes

Regulating EP mediated signaling is essential to allow for adequate repair following injury. Excessive EP2 signaling can increase cytokine production and lead to dysregulated inflammation. PGE_2-EP2 signaling has been shown to impede blood-brain-barrier integrity and exacerbate hippocampal injury induced by seizures by increasing oxidative stress and inflammatory cytokine and chemokine production, which was reversed by EP2 antagonism (12). EP2 blockade also reduced markers of gliosis and neuronal injury that correlated with improvements in behavior scores post-injury (12). Further, following spinal cord injury, there is increased COX-2, mPGES-1 and PGE_2 content in astrocytes and astrocyte-mediated PGE_2 secretion increased production of pro-inflammatory cytokines IL-1β, and IL-6 in macrophages via EP2, which was inhibited by antagonizing EP2 or blocking COX-2 enzyme function (33). Conversely, in human epidermal keratinocytes, EP3 agonism reduced cytokine production following toll-like receptor stimulation, which is thought to influence symptom severity in patients with toxic epidermal necrolysis, as there is typically reduced EP3 expression and concomitant chronic inflammation in conjunctival epithelial tissue of patients (16). While cytokine production is generally necessary during an injury response, having excessive or dysregulated inflammation can exacerbate tissue damage and lead to inadequate repair. Modifying EP signaling to alter cytokine production may prove to be a valuable therapeutic option for highly inflamed tissues.

Modulation of PGE_2 concentration

While PGE_2 signaling has been found to be critical for adequate wound healing to occur in some injury models, there have also been situations where PGE_2 signaling was detrimental to the healing response. In radiation-induced salivary gland damage, elevated PGE_2 levels correlated with salivary gland dysfunction, while reduced PGE_2 levels correlated with improved salivary

gland function post-damage (6). In an impaired wound healing model utilizing LIGHT$^{-/-}$ mice, PGE_2 levels were greatly elevated days 1-7 post dermal excisional wounding when compared to wildtype mice, which correlated with increased elastase activity, platelet aggregation, and reduced hemostasis following injury (5). In a rodent model of tendinopathy, PGE_2 treatment increased the activity of the osteoblast marker alkaline phosphatase, leading to increased ossification of tendon-derived stem cells, which is indicative of poorer recovery. Interestingly, treatment with the COX-2 selective inhibitor, celecoxib attenuated this phenotype and improved tendon repair post-injury (34). Further, reducing PGE_2 levels via celecoxib treatment improved pressure ulcer healing by reducing iNOS, hydroperoxidase, matrix metalloprotease-1 and TNF-α levels. Additionally, celecoxib treatment improved reendothelialization, shortened time to wound closure and strengthened scar formation post-injury (22). These studies suggest that fine-tuning of PGE_2 signaling is necessary for sufficient healing to occur and that dysregulation of PGE_2-mediated inflammation can impair healing following an injury. More detailed descriptions of the studies outlined above can be found in Table 1.

Conclusions

Taken together, these studies illustrate that PGE_2 is a crucial eicosanoid in all three phases of the wound healing response. Regulated PGE_2 signaling is important for proper repair to occur and treatment with non-steroidal anti-inflammatory drugs may be detrimental to a healing tissue. More clearly deciphering the roles of upstream mediators of PGE_2 production, including PLA_2, COX-1, COX-2, mPGES-1, mPGES-2, and cPGES with future studies will help delinate the inflammatory pathways leading to elevated PGE_2 levels and provide more direct therapeutic targets for modulating PGE_2 production. Additionally, defining the regulation and activity of the PGE_2 inactivating enzyme, 15-PGDH, will more precisely outline the timeframe that PGE_2 is able to induce EP-mediated signaling and may unveil opportunities to activate or inhibit 15-

PGDH to improve wound resolution. A better understanding of these components of the PGE_2 pathway will allow for more precise drug targeting to modify PGE_2-induced signaling.

EP receptor signaling plays a major role in dictating the transition from pro- to anti-inflammatory state, either allowing for or inhibiting completion of wound healing. The physiological outputs of EP receptor activation have been more clearly defined for EP2 and EP4 receptors, while the importance of EP1 and EP3 signaling is insufficiently studied in the context of wound healing (outlined in Figure 1). Uncovering different components that regulate EP receptor distribution, such as signaling components that modulate transcription and/or translation, would be highly useful to better understand the importance of each EP receptor subtype. Lastly, a clearer understanding how EP-receptor activation is PGE_2 concentration-dependent across isoforms and tissue types could help guide the use of NSAIDs or 15-PGDH inhibitors to modulate PGE_2-mediated outputs. Further evaluation of EP-specific signaling and generation of EP-selective agonists and antagonists may provide novel therapeutic treatment options to enhance wound recovery following an injury.

Acknowledgments

The authors are supported by the National Institute of Dental and Craniofacial Research grants R01DE029166 (to K. H. Limesand) and F31DE028737 (to K. E. Gilman). The funding agency had no role in preparation of this manuscript. The authors declare no conflicts of interest, financial or otherwise.

Figure Legends:

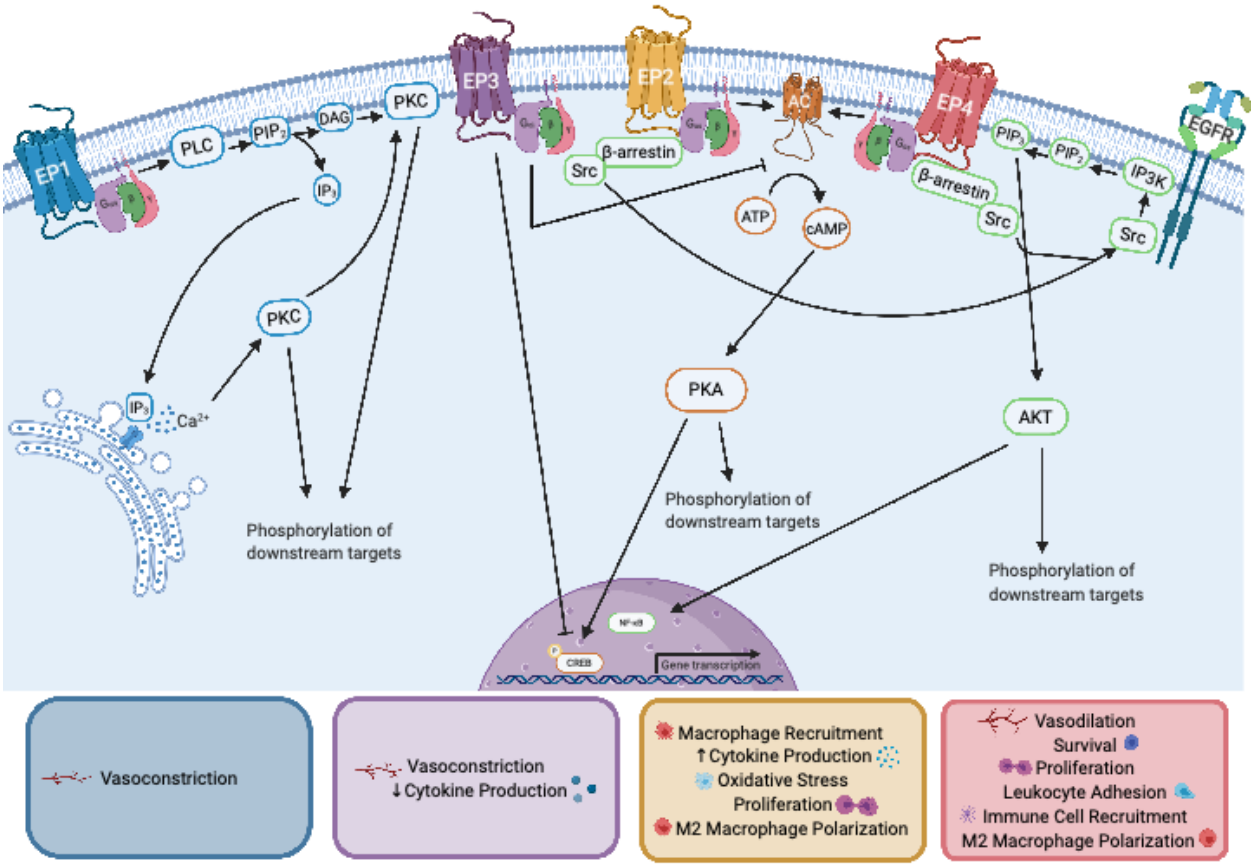

Figure 1. EP receptor signaling and PGE_2-mediated wound healing phenotypes. EP1 (blue) associates with G_q G-protein, which activates phospholipase C (PLC), leading to cleavage of phosphatidylinositol 4,5-bisphosphate (PIP_2), increasing intracellular levels of diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). IP_3 binding to IP_3-gated calcium channels on the endoplasmic reticulum induces release of calcium into the cytosol, which acts in concert with DAG to activate protein kinase C (PKC), leading to phosphorylation of various proteins. EP1 regulated wound healing outputs are listed in the blue box. EP2 (yellow) and EP4 (red) activate two identical signaling pathways. First, G_s g-protein activates adenylate cyclase (AC, orange), which increases intracellular cyclic adenosine monophosphate (cAMP), activating protein kinase A (PKA), leading to phosphorylation of downstream targets including the transcription factor cAMP response element binding protein (CREB). The second pathway (green) occurs via β-arrestin/Src induced EGFR transactivation. This activates IP_3 kinase (IP3K)

315 to phosphorylate PIP_2 to become phosphatidylinositol 3,4,5-trisphosphate (PIP_3), which recruits
316 Akt to the plasma membrane to be phosphorylated and activated. Activated Akt phosphorylates
317 various downstream targets and often induces NF-κB nuclear translocation. EP2 regulated
318 wound healing outputs are listed in the yellow box. EP4 regulated wound healing outputs are
319 listed in the red box. EP3 (purple) associates with the inhibitory g-protein, G_i, blocking the
320 activity of AC and reducing intracellular cAMP levels. EP3 regulated wound healing outputs are
321 listed in the purple box.
322

Model	EPR	[PGE_2]	Major findings	Ref
PGE_2 synthesis or signaling in humans				
Ex vivo Human Cerebral parenchymal arterioles	EP1 EP4	1-10μM PGE_2 1-100nM PGE_2	• Low PGE_2 doses (1-100nM) induce vasodilation while high PGE_2 doses (1-10μM) induce vasoconstriction. • Vasodilation was inhibited by EP4 antagonism (BGC 20-1531, 1μM). • Vasoconstriction was inhibited by EP1 antagonism (SC-51322, 1μM).	(3)
In vitro Human Acute epidermal inflammation	EP3	100μg/mL PGE_2	• PGE_2 treatment (100μg/mL) decreased CCL5, CXCL10 and IL-6 transcription and secretion induced by toll-like receptor activation with Poly(I:C). • EP3 agonism (ONO-AE-248, 10ug/mL) decreased CCL5, CXCL10 and IL-6 transcription and secretion induced by toll-like receptor activation with Poly(I:C).	(16)
Effects of PGE_2 signaling on cell physiology				
Ex vivo *In vivo* Mouse Renal arterial pressure	EP3 EP2/EP4	0.3μM PGE_2	• Administration of PGE_2 (0.3μM) induced vasoconstriction. • EP3 deficient mice exhibited vasodilation following PGE_2 treatment. • EP2 (PF-04418948, 10μM) or EP4 antagonism (L-161982, 1μM) induced vasoconstriction following PGE_2 treatment. • Lower PGE_2 doses (0.001-0.3μM) minimally altered arterial pressure and higher PGE_2 doses (10-30μM) did not exacerbate pressure changes.	(13)
In vitro Rat tendon-derived stem cells (TDSCs)	Not evaluated	50 ng/mL PGE_2	• PGE_2 treatment (50ng/mL) induced osteogenic differentiation, measured by alkaline phosphatase activity, calcium deposition and increased content of osteogenic proteins BMP2, Runx2, OSX and OCN.	(34)

In vitro human-derived enteroid monolayers	Not evaluated	1μM PGE_2	• PGE_2 treatment (1μM) increased intestinal mucus production.	(2)
In vivo Zebrafish DSS-induced colitis	Not evaluated	0.1-10μM PGE_2	• PGE_2 treatment (0.1-10μM) increased intestinal mucus production in a dose-dependent fashion, indicative of intestinal differentiation and improved barrier integrity and survival	(2)
In vitro Mouse Bone marrow derived macrophage (BMDM) activation	Not evaluated	10μM PGE_2	• PGE_2 treatment (10μM) to IL-4-activated M2 macrophages reduced cytoplasmic and mitochondrial reactive oxygen species generation and mitochondrial membrane potential. • PGE_2 treatment (10μM) reduced metabolism and ATP levels due to reduced transcription of malate-aspartate shuttle components.	(23)
In vitro *In vivo* Mouse BMDM activation	Not evaluated	10nM PGE_2	• PGE_2 (10nM) and IL-4 cotreatment enhanced M2 macrophage polarization, indicated by increased M2 gene markers, *Arg1, Mrc1, Fizz1* and *Ym1* that was mediated by CREB induced upregulation of KLF4.	(15)
In vivo Mouse Ear-hole punch injury and macrophage polarization	EP3/EP4	Not quantified	• EP3 (ONO-AE-248, 50nmol/day) or EP4 (ONO-AE-329, 50nmol/day) agonism increased LYVE-1 and VEGFR3 mRNA in wounds • EP3 or EP4 deficient mice exhibited slower wound healing and had reduced LYVE-1, VEGFR3, VEGF-C and VEGF-D mRNA content along with a reduction in macrophage recruitment and reduced M2 markers	(11)
PGE_2 signaling leading to promotion of wound healing				
In vivo Mouse Myocardial infarction (MI) induced injury	EP2	Not quantified	• EP2 deficient mice exhibit poorer heart function and increased infarct size post-MI that correlates with reduced IL-1β, IL-17A, and IL-18 content and reduced macrophage recruitment to the damaged site. • EP2 signaling led to macrophage migration through downregulation of erythroid differentiation regulator 1 (*Erdr1*); macrophage migration defect was rescued via *Erdr1* knockdown in $EP2^{-/-}$ macrophages.	(27)
In vivo Mouse *In vitro* Human DSS-induced colitis	EP4	20μM PGE_2	• Colitis tissue from humans and mice have increased COX-2, but reduced COX-1, and EP4 mRNA and reduced PGE_2 content compared to healthy controls. • PGE_2 treatment (20μM) to mice reduced disease index and apoptosis while increasing proliferation of colon tissue. • PGE_2 treatment (20μM) upregulated EP4 and increased β-arrestin-PI3K-Akt signaling.	(20)

In vivo Zebrafish Tailfin amputation	EP4	0.001-1µM PGE_2	• Reducing endogenous PGE_2 production in zebrafish elevated neutrophil counts at the site of tailfin injury 8-24 hours post damage. • Treating *ptges* deficient zebrafish with PGE_2 (0.001-1µM) reduced neutrophil numbers • Concomitant EP4 antagonism (AH23848, 1µM) and PGE_2 treatment to *ptges* deficient zebrafish elevated neutrophil counts at the injured site	(14)
In vivo Mouse Angiotensin II (Ang II)-induced vascular damage	EP4	Not quantified	• Mice deficient in EP4 on vascular smooth muscle cells (VSMC-$EP4^{-/-}$) are significantly more susceptible to Ang II-induced aortic dissection. • Ang-II treated VSMC-$EP4^{-/-}$ mice have increased aortic size, elastin degradation, oxidative stress, immune infiltration, and matrix metalloprotease -2 and-9 activity. • VSMC-$EP4^{-/-}$ mice had increased vasoconstriction and higher blood pressure compared to WT mice following Ang II-infusion.	(29)
In vivo Mouse Angioplasty wire injury-induced vascular damage	EP2/EP4	100µg/kg misoprostol (a PGE analog)	• Mice deficient in microsomal PGE synthase-1 have elevated neointimal formation and increased immune infiltration 28-days post injury that correlates with reduced PGE_2 content in urine. • Global loss of mPGES-1 reduced reendothelialization that was rescued by treatment with misoprostol, a PGE analog (100µg/kg), or by EP4 agonism (ONO-AE1-329, 1µM). • Endothelial cell proliferation was mediated by EP4-cAMP-PKA signaling. • Endothelial specific deletion of EP4 blocked tissue repair and increased immune infiltration post-injury. • Misoprostol or EP4 agonism following injury improved endothelial repair and reduced neointima formation.	(9)
In vivo Mouse Bone marrow alterations/ efficiency of bone marrow transplantation	EP2/EP4	2-10ng/mg protein PGE_2	• 15-PGDH deficient mice or mice treated with a 15-PGDH inhibitor (SW033291,10mg/kg) have elevated PGE_2 content in bone marrow that was significantly higher than other prostaglandins measured. • 15-PGDH inhibition enhanced hematopoiesis, colony formation and immune cell homing following bone marrow transplantation that could be impeded by antagonizing EP2 (10µg/mouse) or EP4 (10µg/mouse).	(32)
In vivo Mouse DSS-induced colitis	Not evaluated	10-30ng/mg protein PGE_2	• 15-PGDH deficient mice or mice treated with a 15-PGDH inhibitor (SW033291,10mg/kg) have elevated PGE_2 content in colon.	(32)

			• 15-PGDH blockade (15-PGDH$^{-/-}$ mice or SW033291 at 5 or 10mg/kg/twice/day) reversed histological markers of colitis and increased intestinal proliferation after 7 days of DSS treatment.	
In vivo Mouse Hepatectomy-induced liver regeneration	Not evaluated	5-20ng/mg protein PGE_2	• 15-PGDH deficient mice or mice treated with a 15-PGDH inhibitor (SW033291,10mg/kg) have elevated PGE_2 content in liver. • Inhibiting 15-PGDH (15-PGDH$^{-/-}$ or SW033291, 5mg/kg/twice/day) increased liver regeneration by increasing proliferation at day 2 post-surgery that increased liver weight through day 7.	(32)
In vivo Mouse Ear-hole punch injury and macrophage polarization	EP3/EP4	Not quantified	• Ear hole punch injury induced COX-2 and mPGES-1 expression that correlated with lymphangiogenesis and angiogenesis of wounds. • Celecoxib (100mg/kg/day) treatment reduced lymph vessel formation and decreased mRNA content of LYVE-1, VEGFR3, VEGF-C and VEGF-D at day 5. • Celecoxib treatment decreased macrophage recruitment and increased the ratio of M1:M2 polarized macrophages at injured sites. • EP3 (ONO-AE-248, 50nmol/day) or EP4 (ONO-AE-329, 50nmol/day) agonism increased LYVE-1 and VEGFR3 mRNA in wounds • EP3 or EP4 deficient mice exhibited slower wound healing and had reduced LYVE-1, VEGFR3, VEGF-C and VEGF-D mRNA content along with reduced macrophage recruitment and M2 markers.	(11)
In vivo Mouse Mechanical loading and stress fractured bone	Not evaluated	0.75 - 1.25 pg/mL PGE_2 in serum	• Treatment with the non-steroidal anti-inflammatory drug, naproxen (10.9 mg/kg/day) led to worsened stress fracture healing, poorer bone remodeling and increased bones fractures due to reduced toughness. • Naproxen treatment altered fibril formation, reduced 4-hydroxyproline content and led to reduced cortical bone formation that correlated with reduced serum PGE_2 levels.	(18)
In vivo Zebrafish DSS-induced colitis	Not evaluated	0.1-10µM PGE_2	• Concomitant or post-DSS PGE_2 treatment (0.1-10µM) improved survival and reduced intestinal *E.coli* uptake in zebrafish. • PGE_2 increased cell viability and autophagy induction in the zebrafish intestine 5h post-DSS removal.	(2)
In vivo Mouse Skin excision wounding	Not evaluated	0.5-2µM; 0-1ng/mL PGE_2	• Hydrogels incorporated with PGE_2 improved closure of skin wounds in mice.	(31)

In vitro Macrophage polarization			• The proliferation rate of macrophages was altered by PGE_2 dose, with the highest rate observed following 1μM PGE_2 treatment. • Macrophages cultured in PGE_2 containing hydrogels expressed M2 macrophage markers CD68, CD206, IL-10, IL-1Ra and arginase, and had increased mRNA content of angiogenic factors and reduced M1 macrophages markers IL-1β, IL-6 and TNF-α. • Mice receiving PGE_2-incorporated hydrogels had improved wound closure and reduced skin fibrosis at day 14 post-injury.	
In vivo Mouse Bleomycin-induced pulmonary fibrosis	Not evaluated	Not quantified	• Pulmonary fibrosis was reversed with 15-PGDH inhibition (SW033291, 5mg/kg/twice/day). • Inflammatory markers in serum and lung were reduced by 15-PGDH blockade at day-7 post lung injury that correlated with reduced fibrosis, improved airway function and increased survival at day 35.	(24)
In vitro Mouse BMDM activation	Not evaluated	10μM PGE_2	• PGE_2 treatment (10μM) to IL-4-activated M2 macrophages reduced cytoplasmic and mitochondrial reactive oxygen species generation and mitochondrial membrane potential, leading to a reduction in metabolism and ATP levels due to reduced transcription of malate-aspartate shuttle components.	(23)
In vitro *In vivo* Mouse BMDM activation	Not evaluated	10nM PGE_2	• PGE_2 (10nM) and IL-4 cotreatment enhanced M2 macrophage polarization, indicated by increased M2 gene markers, *Arg1, Mrc1, Fizz1* and *Ym1* that was mediated by CREB induced upregulation of KLF4.	(15)
PGE_2 signaling leading to inhibition of wound healing				
In vivo Mouse Seizure-induced neuronal inflammation	EP2	Not quantified	• Seizure-induced injury increased inflammatory cytokines and chemokines (IL-1β, IL-6, TNF-α , CCL2, CCL3, CCL4) that were reduced by EP2 antagonism (TG6-10-1, 5mg/kg). • EP2 blockade dampened gliosis and neuronal injury, preserved blood brain barrier integrity and improved behavioral scores.	(12)
In vivo Rat Spinal cord contusion injury	EP2	200-1000 pg/mg protein; 100-6000 pg/mL media; 0.1-10μM PGE_2	• Astrocytes have increased levels of COX-2, mPGES-1 and PGE_2 due to macrophage migration inhibitory factor activation of JNK-ERK-p38 signaling. • PGE_2 (2.5μM) or astrocyte-conditioned media with simultaneous LPS treatment to primary macrophages increased IL-1β, IL-6 and decreased TNF-α content in supernatant and cell lysates.	(33)

			• EP2 antagonism (PF-04418948, 10μM) or COX-2 inhibition (NS389, 30μM) modulated IL-1β, IL-6 and TNF-α content.	
In vivo *In vitro* Rat Achilles tendinopathy	Not evaluated	50 ng/mL PGE_2	• 12-weeks post-injury, Achilles tendons exhibit ossification and increased OSX and Runx2 staining. • Treatment with COX-2 inhibitor, celecoxib (10mg/kg), immediately following tenotomy reduced ossification that correlated with reduced Akt phosphorylation.	(34)
In vivo Mouse Cutaneous pressure ulcer wounding	Not evaluated	200-500 pg/mg tissue PGE_2	• Ulceration of cutaneous tissue increased COX-1, COX-2, PGE_2, TNF-α, iNOS and lipid hydroperoxide content and induced immune cell recruitment to the wound days 3-7 post-injury. • Treatment with a COX-2 inhibitor, celecoxib (5mg/kg/day), beginning 1 day prior to injury decreased iNOS, TNF-α lipid hydroxyperoxide, COX-2 and PGE_2, improved re-epithelialization markers, and reduced scar formation by day 14.	(22)
In vivo Mouse DSS-induced colitis	Not evaluated	35-75 pg/mg tissue PGE_2	• PGE_2 content increased ~2-fold from 4-7 days post initiation of DSS treatment. • PLA_2 and COX-2 expression levels were elevated 16.6-fold and 7-fold, respectively, at day 4 of DSS treatment. • Elevated PGE_2 levels correlated with reduced colon length and worsened fecal and histological scores.	(8)
In vitro *In vivo* Mouse Radiation-induced salivary gland damage	Not evaluated	200-16,000 pg/mL media PGE_2	• Primary parotid gland cells treated with radiation significantly increase PGE_2 secretion, which correlates with reduced saliva secretion days 3-30 following radiation. • Mice deficient in the extracellular ATP-gated P2X7 receptor secrete significantly less PGE_2 from primary parotid gland cells which correlates with improved saliva secretion days 3-30 following radiation. • Parotid gland tissues treated with radiation increased COX-1 and COX-2, and decreased mPGES-2 and cPGES expression. COX-1 and COX-2 activity remain unchanged. • Parotid gland tissues from $P2X7R^{-/-}$ mice treated with radiation decreased COX-1, mPGES-1, mPGES-2 and cPGES expression. COX-1 activity was increased with treatment.	(6)
In vivo Mouse Impaired dermal excisional	Not evaluated	Relative values; 2-6-fold increase in WT,	• WT mice exhibit a 2-6-fold increase in PGE_2 days 1-3 post-injury. • $LIGHT^{-/-}$ mice (impaired wound healing model) exhibit 1.3-1.8-fold increase in PGE_2 content	(5)

wound healing		1.3-1.8-fold increase in LIGHT$^{-/-}$	days 1-3, with sustained elevated levels at day 7 compared to WT mice. • Elevated PGE_2 levels in LIGHT$^{-/-}$ mice correlated with elevated elastase activity, platelet aggregation and reduced hemostasis.	
In vitro *In vivo* Mouse BMDM activation and high fat feeding induced insulin resistance	Not evaluated	10nM PGE_2	• Myeloid-specific CREB knockdown increased M1 macrophage markers and macrophage infiltration in white adipose tissue and induced systemic insulin resistance during high-fat feeding in mice.	(15)

Table 1. PGE_2-EP receptor mediated wound healing phenotypes in different damage models.

References:

1. **Buchanan FG, Wang D, Bargiacchi F, and DuBois RN**. Prostaglandin E2 Regulates Cell Migration via the Intracellular Activation of the Epidermal Growth Factor Receptor. *J Biol Chem* 278: 35451-35457, 2003.

2. **Chuang L-S, Morrison J, Hsu N-Y, Labrias PR, Nayar S, Chen E, Villaverde N, Facey JA, Boschetti G, Giri M, Castillo-Martin M, Thin TH, Sharma Y, Chu J, and Cho JH**. Zebrafish modeling of intestinal injury, bacterial exposures and medications defines epithelial in vivo responses relevant to human inflammatory bowel disease. *Dis Model Mech* 12: dmm037432, 2019.

3. **Czigler A, Toth L, Szarka N, Szilágyi K, Kellermayer Z, Harci A, Vecsernyes M, Ungvari Z, Szolics A, Koller A, Buki A, and Toth P**. Prostaglandin E(2,) a postulated mediator of neurovascular coupling, at low concentrations dilates whereas at higher concentrations constricts human cerebral parenchymal arterioles. *Prostaglandins Other Lipid Mediat* 146: 106389, 2020.

4. **Dennis EA, and Norris PC**. Eicosanoid Storm in Infection and Inflammation. *Nature reviews Immunology* 15: 511-523, 2015.

5. **Dhall S, Wijesinghe DS, Karim ZA, Castro A, Vemana HP, Khasawneh FT, Chalfant CE, and Martins-Green M**. Arachidonic acid-derived signaling lipids and functions in impaired healing. *Wound Repair Regen* 23: 644-656, 2015.

6. **Gilman KE, Camden JM, Klein RR, Zhang Q, Weisman GA, and Limesand KH**. P2X7 receptor deletion suppresses γ-radiation-induced hyposalivation. *Am J Physiol Regul Integr Comp Physiol* 2019.

7. **Gonzalez ACdO, Costa TF, Andrade ZdA, and Medrado ARAP**. Wound healing - A literature review. *An Bras Dermatol* 91: 614-620, 2016.

8. **Hamabata T, Nakamura T, Masuko S, Maeda S, and Murata T**. Production of lipid mediators across different disease stages of dextran sodium sulfate-induced colitis in mice. *J Lipid Res* 59: 586-595, 2018.

9. **Hao H, Hu S, Wan Q, Xu C, Chen H, Zhu L, Xu Z, Meng J, Breyer RM, Li N, Liu D-P, FitzGerald GA, and Wang M**. A Protective Role of Microsomal Prostaglandin E Synthase-1 Derived PGE2 and the Endothelial EP4 Receptor in Vascular Responses to Injury. *Arteriosclerosis, thrombosis, and vascular biology* 38: 1115-1124, 2018.

10. **Hata AN, and Breyer RM**. Pharmacology and signaling of prostaglandin receptors: Multiple roles in inflammation and immune modulation. *Pharmacology & Therapeutics* 103: 147-166, 2004.

11. **Hosono K, Isonaka R, Kawakami T, Narumiya S, and Majima M**. Signaling of Prostaglandin E Receptors, EP3 and EP4 Facilitates Wound Healing and Lymphangiogenesis with Enhanced Recruitment of M2 Macrophages in Mice. *PLOS ONE* 11: e0162532-e0162532, 2016.

12. **Jiang J, Yu Y, Kinjo ER, Du Y, Nguyen HP, and Dingledine R**. Suppressing pro-inflammatory prostaglandin signaling attenuates excitotoxicity-associated neuronal inflammation and injury. *Neuropharmacology* 149: 149-160, 2019.

13. **Liu B, Wu X, Zeng R, Yin Y, Guo T, Xu Y, Zhang Y, Leng J, Ge J, Yu G, Guo J, and Zhou Y**. Prostaglandin E(2) sequentially activates E-prostanoid receptor-3 and thromboxane prostanoid

receptor to evoke contraction and increase in resistance of the mouse renal vasculature. *Faseb j* 34: 2568-2578, 2020.

14. **Loynes CA, Lee JA, Robertson AL, Steel MJ, Ellett F, Feng Y, Levy BD, Whyte MKB, and Renshaw SA**. PGE(2) production at sites of tissue injury promotes an anti-inflammatory neutrophil phenotype and determines the outcome of inflammation resolution in vivo. *Sci Adv* 4: eaar8320-eaar8320, 2018.

15. **Luan B, Yoon Y-S, Lay JL, Kaestner KH, Hedrick S, and Montminy M**. CREB pathway links PGE2 signaling with macrophage polarization. *PNAS* 112: 15642-15647, 2015.

16. **Mieno H, Ueta M, Yamada K, Yamanaka Y, Nakayama T, Watanabe A, Kinoshita S, and Sotozono C**. Expression of prostaglandin E(2) receptor 3 in the eyelid epidermis of patients with Stevens-Johnson syndrome/toxic epidermal necrolysis. *Br J Ophthalmol* 2019.

17. **Nakanishi M, and Rosenberg DW**. Multifaceted roles of PGE2 in inflammation and cancer. *Semin Immunopathol* 35: 123-137, 2013.

18. **Park J, Fertala A, and Tomlinson RE**. Naproxen impairs load-induced bone formation, reduces bone toughness, and diminishes woven bone formation following stress fracture in mice. *Bone* 124: 22-32, 2019.

19. **Park JY, Pillinger MH, and Abramson SB**. Prostaglandin E2 synthesis and secretion: The role of PGE2 synthases. *Clinical Immunology* 119: 229-240, 2006.

20. **Peng X, Li J, Tan S, Xu M, Tao J, Jiang J, Liu H, and Wu B**. COX-1/PGE2/EP4 alleviates mucosal injury by upregulating beta-arr1-mediated Akt signaling in colitis. *Sci Rep* 7: 1055, 2017.

21. **Ricciotti E, and FitzGerald GA**. Prostaglandins and Inflammation. *Arteriosclerosis, thrombosis, and vascular biology* 31: 986-1000, 2011.

22. **Romana-Souza B, Santos JS, Bandeira LG, and Monte-Alto-Costa A**. Selective inhibition of COX-2 improves cutaneous wound healing of pressure ulcers in mice through reduction of iNOS expression. *Life Sci* 153: 82-92, 2016.

23. **Sanin DE, Matsushita M, Klein Geltink RI, Grzes KM, van Teijlingen Bakker N, Corrado M, Kabat AM, Buck MD, Qiu J, Lawless SJ, Cameron AM, Villa M, Baixauli F, Patterson AE, Hässler F, Curtis JD, O'Neill CM, O'Sullivan D, Wu D, Mittler G, Huang SC, Pearce EL, and Pearce EJ**. Mitochondrial Membrane Potential Regulates Nuclear Gene Expression in Macrophages Exposed to Prostaglandin E2. *Immunity* 49: 1021-1033.e1026, 2018.

24. **Smith JNP, Witkin MD, Jogasuria AP, Christo KF, Raffay TM, Markowitz SD, and Desai AB**. Therapeutic targeting of 15-PGDH in murine pulmonary fibrosis. *Scientific Reports* 10: 11657, 2020.

25. **Sugimoto Y, and Narumiya S**. Prostaglandin E receptors. *J Biol Chem* 282: 11613-11617, 2007.

26. **Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist PH, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, and Pontén F**. Proteomics. Tissue-based map of the human proteome. *Science* 347: 1260419, 2015.

27. **Wu JMF, Cheng YY, Tang TWH, Shih C, Chen JH, and Hsieh PCH**. Prostaglandin E(2) Receptor 2 Modulates Macrophage Activity for Cardiac Repair. *J Am Heart Assoc* 7: e009216, 2018.
28. **Xu C, Fu F, Li X, and Zhang S**. Mesenchymal stem cells maintain the microenvironment of central nervous system by regulating the polarization of macrophages/microglia after traumatic brain injury. *Int J Neurosci* 127: 1124-1135, 2017.
29. **Xu H, Du S, Fang B, Li C, Jia X, Zheng S, Wang S, Li Q, Su W, Wang N, Zheng F, Chen L, Zhang X, Gustafsson J-Å, and Guan Y**. VSMC-specific EP4 deletion exacerbates angiotensin II-induced aortic dissection by increasing vascular inflammation and blood pressure. *Proceedings of the National Academy of Sciences of the United States of America* 116: 8457-8462, 2019.
30. **Yao L, Chen W, Song K, Han C, Gandhi CR, Lim K, and Wu T**. 15-hydroxyprostaglandin dehydrogenase (15-PGDH) prevents lipopolysaccharide (LPS)-induced acute liver injury. *PLOS ONE* 12: e0176106-e0176106, 2017.
31. **Zhang S, Liu Y, Zhang X, Zhu D, Qi X, Cao X, Fang Y, Che Y, Han Z-C, He Z-X, Han Z, and Li Z**. Prostaglandin E(2) hydrogel improves cutaneous wound healing via M2 macrophages polarization. *Theranostics* 8: 5348-5361, 2018.
32. **Zhang Y, Desai A, Yang SY, Bae KB, Antczak MI, Fink SP, Tiwari S, Willis JE, Williams NS, Dawson DM, Wald D, Chen WD, Wang Z, Kasturi L, Larusch GA, He L, Cominelli F, Di Martino L, Djuric Z, Milne GL, Chance M, Sanabria J, Dealwis C, Mikkola D, Naidoo J, Wei S, Tai HH, Gerson SL, Ready JM, Posner B, Willson JK, and Markowitz SD**. TISSUE REGENERATION. Inhibition of the prostaglandin-degrading enzyme 15-PGDH potentiates tissue regeneration. *Science* 348: aaa2340, 2015.
33. **Zhang Y, Zhou Y, Chen S, Hu Y, Zhu Z, Wang Y, Du N, Song T, Yang Y, Guo A, and Wang Y**. Macrophage migration inhibitory factor facilitates prostaglandin E(2) production of astrocytes to tune inflammatory milieu following spinal cord injury. *J Neuroinflammation* 16: 85-85, 2019.
34. **Zhou W, Lin X, Chu J, Jiang T, Zhao H, Yan B, and Zhang Z**. Magnolol prevents ossified tendinopathy by inhibiting PGE2-induced osteogenic differentiation of TDSCs. *Int Immunopharmacol* 70: 117-124, 2019.