P2 purinergic receptor dysregulation in urologic disease

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Abstract
P2 purinergic receptors are involved in the normal function of the kidney, bladder, and prostate via signaling that occurs in response to extracellular nucleotides. Dysregulation of these receptors is common in pathological states and often associated with disease initiation, progression, or aggressiveness. Indeed, P2 purinergic receptor expression is altered across multiple urologic disorders including chronic kidney disease, polycystic kidney disease, interstitial cystitis, urinary incontinence, overactive bladder syndrome, prostatitis, and benign prostatic hyperplasia. P2 purinergic receptors are likewise indirectly associated with these disorders via receptor-mediated inflammation and pain, a common characteristic across most urologic disorders. Furthermore, select P2 purinergic receptors are overexpressed in urologic cancer including renal cell carcinoma, urothelial carcinoma, and prostate adenocarcinoma, and pre-clinical studies depict P2 purinergic receptors as potential therapeutic targets. Herein, we highlight the compelling evidence for the exploration of P2 purinergic receptors as biomarkers and therapeutic targets in urologic cancers and other urologic disease. Likewise, there is currently optimism for P2 purinergic receptor-targeted therapeutics for the treatment of inflammation and pain associated with urologic diseases. Further exploration of the common pathways linking P2 purinergic receptor dysregulation to urologic disease might ultimately help in gaining new mechanistic insight into disease processes and therapeutic targeting.

Keywords Purinergic receptors · P2X · P2Y · Urologic disease · Extracellular ATP · Prostate cancer

Janielle P. Maynard is an Assistant Professor of pathology and oncology at the Johns Hopkins University School of Medicine. Dr. Maynard’s research interests involve the investigation of inflammation-associated P2 purinergic receptors and their role in cancer initiation and aggressiveness. Specifically, her work determined that P2X3 purinergic receptor overexpression is associated with lower recurrence-free survival in patients with hepatocellular carcinoma and identified P2X4 purinergic receptors as overexpressed in prostate cancer compared to benign tissues, in cases with common genetic alterations, and as associated with increased risk of metastasis. Studies are aimed at therapeutically targeting P2 purinergic receptors in patients with aggressive cancer. Dr. Maynard’s research also involves the investigation of immunobiological factors that contribute to cancer health disparities in racial/ethnic minorities and underserved populations. Dr. Maynard graduated with Bachelor of Science degrees in Biology and Chemistry from Howard University. She earned her Ph.D. in Translational Biology and Molecular Medicine from Baylor College of Medicine. Dr. Maynard joined the Department of Pathology at the Johns Hopkins University School of Medicine as a Postdoctoral Fellow and later joined the faculty.
The typical consequence is facilitates pore opening through which cations freely move. This transmembrane receptors bind eATP in their extra-
membrane and an increase in the concentration of free cytosolic Ca
response against microbes. [1, 3] Alternatively, P2X3 receptor expression is abundant in primary afferent neurons corresponding to its nociceptive function. [5, 6]

The P2Y receptors are a family of G protein-coupled receptors of which eight mammalian subtypes have been identified. [7] Two subfamilies are described as the Gq-coupled P2Y1-like (P2Y1, P2Y2, P2Y4, P2Y6, and P2Y11) and the Gi-coupled P2Y12-like (P2Y12, P2Y13, P2Y14) receptors. [7] Unlike their P2X counterparts, P2Y receptors are differentially activated by diverse purines and pyrimidines. [7] When activated, P2Y receptors can couple through the inositol phosphate (IP3) pathway thereby mobilizing Ca[2+] from intracellular stores that directly control cell function (Fig. 1). [7] Similar to P2X receptors, P2Y receptors are differentially expressed across cell types and can sometimes only be detected in pathological conditions (Table 1). [1] For instance, P2Y6 receptor is highly expressed in T cells infiltrating active inflammatory bowel disease, but is absent in T cells in the unaffected bowel. [8] Diabetes, Alzheimer’s disease, and cancer are likewise among the pathological conditions that induce P2 purinergic receptor expression. [9–11]

P2 purinergic receptors have diverse functional roles across multiple cell types, including cell proliferation, apoptosis, cell differentiation, immune cell activation and recruitment, and pain transmission. [1] We focus this review on the expression and function of these receptors in the following urologic diseases: chronic kidney disease (CKD), polycystic kidney disease (PKD), kidney cancer, interstitial cystitis (IC), urinary incontinence, overactive bladder syndrome, bladder cancer, prostateitis, benign prostatic hyperplasia (BPH), prostate cancer, erectile dysfunction, and infertility. Among the most common characteristics of urologic diseases are inflammation and pain.

Inflammation and urologic disease

Inflammation both contributes to and can be the consequence of several urologic diseases. There is substantial evidence that inflammation contributes to renal injury as well as the development and progression of CKD. [12] Urinary tract infections are among the most commonly diagnosed bacterial infections and bladder-related disorders such as interstitial cystitis are likewise strongly associated with inflammation. [13, 14] Chronic inflammation is frequently observed in the adult prostate, is proposed to play a causative role in the development of benign prostatic hyperplasia BPH, and is the defining characteristic of prostateitis. [15–17]
Chronic inflammation is also a known or suspected risk factor for many cancers including prostate, bladder, and kidney cancers. Therefore, understanding the mechanisms associated with initiation and propagation of the immune response is essential for improving the management of urologic diseases.

In 1994, Polly Matzinger postulated that the immune system responds to danger signals instead of simply factors that are foreign. While foreign or pathogen-derived signals illicit an immune response, so too could endogenous danger signals released or produced by cells that are injured, stressed, or undergoing cell death. Among the endogenous danger signals is eATP. Considerably greater eATP concentrations are measured in inflamed versus healthy tissues in mouse models of graft-versus-host disease and allergic contact dermatitis. Increased concentrations of eATP act as a powerful chemotactic stimulus for immune cells, many of which express P2 purinergic receptors. Immune cell P2 receptor activation results in chemokine and inflammatory marker induction and the assembly or activation of the inflammasome (Fig. 1). Propagation of inflammation is thought, in part, to upregulate multiple P2 purinergic receptors on both immune and non-immune cells. Consequently, purinergic signaling continues to be studied in chronic inflammation and in inflammation-associated pathologies such as inflammatory pain, rheumatoid arthritis, and glomerulonephritis.

**Pain and urologic disease**

Local and referred pain are common symptoms associated with urologic disorders and are often the motivation for seeking medical attention. Infections contribute to a large portion of these concerns; however, other non-infection mediated chronic disorders are also characterized by pain. For instance, interstitial cystitis/bladder pain syndrome (IC/BPS) and chronic nonbacterial prostatitis/chronic pelvic pain syndrome are both diagnosed after clinical infection, malignancy, and/or other identifiable causes for long-term urological pain are ruled out. These disorders can greatly affect the patient’s quality of life. Similarly, advanced genitourinary cancers are characterized by excruciating pain, particularly after bone metastases. Studying pain associated with urologic disease, whether direct or indirect, is important for the holistic treatment of the patient.

Early purinergic signaling studies identified eATP as a neurotransmitter. Evidence that ATP was released from sensory nerves and consequently elicited action potentials in dorsal root ganglionic (DRG) neurons initiated interest in P2 purinergic receptors for their role in pain processing. Since then, P2X3 and P2X2/3 receptors were identified as key facilitators of direct neuronal activation (Fig. 1). Activation of other P2 receptors, including P2X4 and P2X7, and some P2Y receptors can modulate pain neurotransmission via indirect mechanisms involving glial-neuronal...
interactions and/or modulation of other nociceptive-specific receptors. \[6\] Due to P2 purinergic receptor involvement in various pain states, P2 receptor antagonism has been investigated for pain management in pre-clinical studies and clinical trials. \[5, 27\] For example, P2X3 antagonist AF-315 produced dose-dependent anti-hyperalgesia in both an adjuvant-induced arthritis rat model and a rat model of knee osteoarthritis. \[5\]

Kidney

P2 purinergic receptor expression in the kidney has been reported in mice, rats, and in human primary mesangial and visceral glomerular epithelial cells (Table 1). \[28–30\] Purinergic signaling is well-studied in the normal function of the kidney, particularly in blood pressure regulation (Fig. 2). For example, P2Y2 receptors play a prominent role in the regulation of renal electrolyte and water transport. \[31\] P2Y2 knockout mice have salt-resistant hypertension associated with abnormal renal Na\(^+\) and fluid physiology. \[32\] Furthermore, a P2Y2 receptor agonist reduced blood pressure and increased renal Na\(^+\) excretion in mice, indicating that these receptors might be potential therapeutic targets. \[33\] P2X4 knockout mice are also hypertensive, but dietary Na\(^+\) restriction normalizes blood pressure in these mice. \[30\] Interestingly, a loss-of-function polymorphism in the human P2X4 receptor is associated with increased pulse pressure, but this polymorphism has not been studied specifically in renal function. \[34\] Altogether, the evidence suggests that P2 purinergic receptors have critical roles in normal kidney function. Here, we discuss how expression and dysregulation of these receptors contribute to kidney disease (Fig. 2).

Chronic kidney disease

CKD is the most common disease of the kidney and affects about 15% of the adult population in the USA. \[35\] Diagnosis is defined by substantially reduced glomerular filtration rate (\(<60\) mL/min/1.73 m\(^2\)) or albuminuria (\(\geq30\) mg per 24 h) for more than 3 months \[36\] and is associated with increased cardiovascular and overall mortality. \[35\] Persistent, low-grade inflammation is considered a hallmark

| Table 1 P2 purinergic receptor expression in urologic tissues |
|-------------|----------------|----------------|
| Tissue      | Species | Receptor expressed | Reference |
| Kidney      | Zebrafish | P2X7            | [52]       |
|             | Mouse   | P2X2, P2X4, P2X7 | [175]      |
|             | Rat     | P2X4, P2X5, P2X7, P2Y1, P2Y2, P2Y4, P2Y6 | [29, 51, 56] |
|             | Human   | P2X6, P2X7       | [29, 62, 63] |
| Ureter      | Human   | P2X4, P2Y1       | [75]       |
| Urethra     | Mouse   | P2Y6            | [76]       |
| Vas deferens | Mouse   | P2X1            | [176]      |
| Seminal vesicle | Mouse | P2X1          | [176]      |
| Epididymis  | Mouse   | P2X1—P2X3       | [176]      |
| Sperm       | Mouse   | P2X1—P2X4       | [177]      |
|             | Rat     | P2X1—P2X3       | [177]      |
|             | Hamster | P2X1—P2X4       | [177]      |
|             | Human   | P2X1—P2X4       | [177]      |
| Uterus      | Mouse   | P2X1            | [176]      |
| Vagina      | Mouse   | P2X1            | [176]      |
| Bladder     | Mouse   | P2X1, P2X4, P2Y1, P2Y2, P2Y6, P2Y12, P2Y14 | [76, 176, 178] |
|             | Rat     | P2X1, P2X3, P2X4, P2X7, P2Y1, P2Y2, P2Y4, P2Y6 | [80, 179] |
| Rabbit      | P2X3    |                | [180]      |
| Cat         | P2X1—P2X7, P2Y1, P2Y2, P2Y4 | 71 |
| Human       | P2X1—P2X7, P2Y1, P2Y2, P2Y6, P2Y11, P2Y12, P2Y13, P2Y14 | [14, 72–75, 83] |
| Prostate    | Mouse   | P2X1            | [89]       |
|             | Rat     | P2X1—P2X7       | [95]       |
|             | Human   | P2X4, P2X7       | [108, 113] |
| Corpora cavernosa | Mouse | P2X1          | [140]      |
|             | Rat     | P2X1, P2X3, P2X4, P2X7, P2Y1, P2Y2, P2Y4, P2Y6 | [137, 138, 179] |
|             | Human   | P2X1, P2Y1, P2Y2, P2Y6 | [128, 129] |
The NLRP3 inflammasome contributes to CKD development and P2X7 is a known activator of this inflammasome. Accordingly, a role for P2 receptors in the inflammatory aspects of CKD has been investigated. P2X7 knockout mice fed a high-fat diet had attenuated renal function, reduced inflammation, fibrosis, and oxidative stress compared to wild type (WT) animals. In this study, WT mice fed a high-fat diet had significantly increased urinary protein/creatinine and albumin/creatinine, induced glomerular hypertrophy determined by periodic acid Schiff staining, increased glomerular cell apoptosis determined by active caspase-3 (2.5-fold), increased fibronectin and collagen IV staining, increased oxidative stress marked by upregulation of Nox4 expression, and increased NLRP3 and pro-IL-1β mRNA expression compared to WT mice fed a normal-fat diet (all \( P < 0.001 \)). All of these parameters were attenuated in P2X7 knockout mice fed a high-fat diet, and this finding was attributed to attenuated NLRP3 inflammasome activation in the P2X7 knockout mice. Others used flow cytometry to show that P2X7 mRNA expression is increased in peripheral blood mononuclear cells (PMBCs) from CKD patients \( (n = 15) \) compared to healthy volunteers \( (n = 15) \) \( (P < 0.05) \) and that P2X7 protein expression is increased specifically on B-lymphocyte \( (P < 0.05) \) and monocyte \( (P < 0.001) \) populations from patients in early-stage CKD. P2X7 receptors are involved in the defective calcium signaling in PMBCs of patients with CKD by facilitating increased intracellular \( \text{Ca}^{2+} \) in these cells. Collectively, these data suggest that P2X7 facilitates CKD pathogenesis. Alternatively, studies indicate that the P2Y2 receptor might serve a protective function. Subtotal nephrectomy (SNX) was performed on P2Y2 knockout mice as a model of CKD. P2Y2 knockout mice had reduced survival after SNX compared to after sham surgery (63.1%, \( n = 19 \) vs 100%, \( n = 9 \); \( P < 0.01 \), respectively), while WT mice had no statistical difference in survival rate (88.9% vs 100%). P2Y2 knockout mice also had increased blood pressure (177 ± 2 mmHg) compared to WT mice (156 ± 7 mmHg) at day 56 post-SNX \( (P < 0.05) \). Finally, P2Y2 knockout mice exhibited greater overall renal injury evidenced by increased serum urea \( (P < 0.05) \) and increased urine albumin-to-creatinine ratio (UACR) (2.5-fold, \( P < 0.05 \)) after SNX compared to WT mice. Acute kidney injury (AKI) is associated with CKD and is a leading cause of mortality or morbidity in hospitalized patients. Patients with severe CKD \( (< 15 \text{ mL/min/1.73 m}^2) \) had a dramatically increased risk of AKI (odds ratio = 47.17; \( CI: 39.22–56.74 \)) compared to non-CKD patients \( (≥ 60 \text{ mL/min/1.73 m}^2) \). Several meta-analysis studies have associated AKI with later development of CKD. A major cause of AKI is renal ischemia and reperfusion (IR) resulting in renal tubular necrosis, inflammation, and apoptosis. Consequently, increased eATP after IR activates P2X4 or P2X7. Activation of P2X4 results in NLRP3 inflammasome induction in renal proximal tubule cells, which exacerbates ischemic AKI. Activation of
P2X7 induces peptidyl arginine deiminases 4 (PAD4), which is known to exacerbate ischemic AKI, via PKC activation in human and mouse renal proximal tubule cells. [42] Excitingly, early P2X7 inhibition protected against IR-induced AKI in a mouse model. [47] Similarly, P2Y14 inhibition or genetic ablation inhibited IR injury–induced chemokine expression and reduced neutrophil renal infiltration, kidney dysfunction, and proximal tubule damage associated with AKI. [48] Concentrations of P2Y14 ligand, UDP-glucose, are elevated in urine samples from patients who develop AKI compared to patients without AKI. [48] These studies identify P2X7 and P2Y14 as potential therapeutic targets for the treatment of AKI.

**Polycystic kidney disease**

Polycystic kidney disease (PKD) is a genetic disorder that afflicts about 500,000 people in the USA [49] and over 10 million people worldwide. [50] PKD is often characterized by hypertension, pain, hepatic fibrosis, and end-stage renal disease. [50] P2Y2, P2Y6, and P2X7 mRNA expression was increased in a rat model of autosomal dominant PKD (ADPKD), the most common form of the disease, compared to WT littermates. [51] Protein expression of each receptor was confirmed by immunohistochemistry (IHC). [51] In a pkd2 morphant ADPKD zebrafish model, P2X7 mRNA expression was observed earlier and more strongly compared to control embryos. [52] Both P2X7 knockdown and inhibition significantly reduced the frequency of cyst formation via ERK-dependent pathways in pkd2 morphants (55.8%, \( P < 0.01 \) and 35.7%, \( P < 0.01 \), respectively). [52] Alternatively, Hillman et al. report that P2X7 activation reduced cystogenesis from cell aggregates derived from an autosomal recessive PKD (ARPKD) mouse model. [53] Enhanced ATP release in human ADPKD is reported as well as in mouse and rat ARPKD cell models, compared to controls. [54, 55] Hillman et al. propose that this excess ATP might serve as a natural brake on cyst formation, while others report that P2X7 and other P2 purinergic receptors facilitate cystogenesis. [51–54, 56] The authors do not discuss this discrepancy. One consideration is the different P2 purinergic profiles observed between ADPKD and ARPKD or across species. For instance, Schiebert et al. showed that human ADPKD primary cells lack P2Y1 but express P2Y2 and P2Y6 while mouse cpk ARPKD primary cells expressed P2Y1 and P2Y2 but not P2Y6 (Table 2). [54] Further studies are necessary to differentiate P2 purinergic receptor profiles and functionality, between ADPKD and ARPKD and to determine which pathways are relevant in human PKD.

**Kidney cancer**

Renal cell carcinoma (RCC) accounts for about 90% of all kidney cancers. [18] RCC is further classified into subtypes including clear cell (~75% of cases), papillary (~15%), and chromophobe (~5%). [57] Transitional cell carcinoma, Wilms tumor, and renal sarcoma make up the remainder of kidney cancers. Altogether, the 5-year survival rate for kidney and

| Table 2 | P2 purinergic receptor expression in urologic cells and cell lines |
|---------|------------------------------------------------------------------------------------------------|
| Tissue  | Cell line | Receptor expressed | Reference |
| Kidney  | SN12-PM6 cells | P2X6 | [63] |
|         | SW839 cells | P2X6 | [63] |
|         | A498 cells | P2X6 | [63] |
|         | ADPKD cells | P2X2, P2X4, P2X5, P2X7 | [54] |
|         | Human glomerular epithelial cells | P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2X4—P2X7 | [28] |
| Bladder | T24 cells | P2X3, P2Y2, P2Y6 | [86, 181] |
|         | 5637 | P2Y2 | [86] |
|         | HT-1376 | P2X4, P2X5, P2X7, P2Y1, P2Y2, P2Y4, P2Y6, P2Y11 | [182] |
| Prostate | PC3 cells | P2X4, P2X5, P2X7, P2Y1, P2Y2 | [105, 106, 108] |
|         | DU145 cells | P2X4, P2X5, P2Y1, P2Y2, P2Y6, P2Y11, P2Y14 | [106, 108] |
|         | LNCaP cells | P2X4, P2X5, P2X7, P2Y2, P2Y4, P2Y6, P2Y12, P2Y13, P2Y14 | [106–108] |
|         | CWR 22Rv1 | P2X4 | [108] |
|         | VCaP | P2X4 | [108] |
|         | LAPC4 | P2X4 | [108] |
|         | MD PCA 2b | P2X4 | [108] |
|         | C4-2 cells | P2X4, P2X7, P2Y11, P2Y12, P2Y14 | [107] |
|         | C4-2B cells | P2X4, P2X7, P2Y1, P2Y4, P2Y11, P2Y12, P2Y13, P2Y14 | [107] |
|         | PNT-2 cells | P2X4, P2X5, P2X7, P2Y1, P2Y2, P2Y4, P2Y6, P2Y11 | [109] |
| Testicle | Testicular peritubular cells | P2X4, P2X7 | [141] |
renal pelvis cancer is about 75%. [58] Currently, surgery is the main treatment for kidney cancer and targeted therapies are often adjuvant therapy after surgery or used to treat advanced disease, with minimal demonstrated benefit. [18]

Purinergic signaling has been studied for both causative and prognostic roles in cancer. Substantially greater eATP concentrations are measured in the tumor microenvironment (TME) of mice compared to healthy tissues and receptor overexpression correlates to poorer outcomes in multiple cancer types. [22, 59–62] In kidney cancer, the average IHC staining score of peritumoral P2X7 expression was significantly higher than intratumoral P2X7 expression ($P < 0.001$). [62] P2X7 expression was dichotomized into high expression (above median, $n = 138$) and low expression (below median, $n = 135$). Kaplan–Meier survival analysis showed that clear-cell renal carcinoma patients with high intratumoral P2X7 expression had worse cancer-specific survival than those with low intratumoral P2X7 expression ($P < 0.001$). [62] Furthermore, prognostic accuracy of TNM stage; the University of California Integrated Staging System (UISS); and the stage, size, grade, and necrosis (SSIGN) scoring models was improved when intratumoral P2X7 expression was added. [62] Another study reported that P2X6 activation drives invasiveness of RCC cells via Ca$^{2+}$-mediated p-ERK1/2/MMP9 signaling. [63]

Interestingly, multiple P2 purinergic receptors are dysregulated in kidney cancer tissues in The Cancer Genome Atlas (TCGA) public gene datasets as assessed via Wanderer. [64–68] For instance, the majority of receptors—P2X1, P2X3, P2X4, P2X7, P2Y1, P2Y4, P2Y6, P2Y13, and P2Y14—were significantly increased in clear cell RCC tumors compared to normal (Fig. 3). Patients with clear cell RCC had the worst survival and a higher expression of immune signature genes in their tumors compared to patients with papillary and chromophobe RCC. [57] Alternatively, the majority of receptors—P2X1, P2X2, P2X5, P2X6, P2Y2, P2Y6, and P2Y14 receptors—were significantly reduced in chromophobe RCC tumors compared to normal (Fig. 3). Interestingly, papillary cell RCC which is described as the heterogeneous subtype had a more balanced P2 receptor expression profile with P2X4, P2X7, and P2Y13 receptors increased and P2X2, P2X6, P2Y1, P2Y2, and P2Y14 receptors decreased in tumors compared to normal (Fig. 3). Such profiles may be indicative of the phenotypes observed across the three types of RCC. Future studies are necessary to investigate the association between P2 purinergic receptor expression profiles and kidney cancer phenotypes and outcomes. There is a need to characterize the expression profiles of individual receptor subtypes, determine their function, and assess whether there is any efficacy in targeting these receptors pharmacologically for the treatment of kidney cancers.

**Bladder, ureter, and urethra**

Release of ATP by urothelium and consequent activation of P2 purinergic receptors are known to be involved in motor and sensory functions of the urinary bladder. [69] Bladder stretch from filling induces cytosolic Ca$^{2+}$ increase, promoting ATP release from the urothelium (Fig. 4). This eATP binds to P2 purinergic receptors to trigger nerve activation, the sensation of bladder fullness, and the urge to urinate. [69, 70] Purinergic signaling contributes to bladder contraction, voiding reflex, urinary urgency, and related pain. [71] P2X1–7, P2Y1, P2Y2, P2Y6, P2Y11, P2Y12, P2Y13, and P2Y14 receptor expressions have each been identified in the human bladder (Table 1). [14, 69]
Under physiologic conditions, P2 purinergic receptor knockout mice appear to have no substantial differences in bladder function compared to WT. However, under pathological conditions, inhibition of P2 purinergic receptors improves bladder hyperactivity. We discuss the expression and function of P2 purinergic receptors in select bladder-related disorders (Fig. 4).

**Interstitial cystitis**

IC/BPS is a chronic, painful inflammatory bladder condition characterized by pelvic pain and urinary symptoms without an identifiable cause. IC/BPS is often under-diagnosed and mistreated, in part because the etiology of the disease is not well-understood. Tissue samples from feline IC urothelium showed reduced P2X1 and P2Y2 expression compared to tissues from normal cats. Alternatively in humans, western blotting analysis revealed significant upregulation of P2X1, P2X2, and P2X3 receptor protein expression in urothelial cells isolated from IC patients (n = 8) compared to age-, race-, and sex-matched asymptomatic control subjects (n = 6) (P < 0.05). Similarly, P2Y1, P2Y2, and P2Y11 receptor mRNA expression was significantly upregulated in urothelium in IC patients (P < 0.05). Stretch-activated release of ATP in cultured bladder urothelial cells from IC patients was higher compared to cells from control patients. In another study, P2X2 and P2X3 protein expression was increased in IC patients compared to control patients in spite of the comparable P2X2 and reduced P2X3 mRNA expression in IC patients. The authors propose that potential negative feedback might account for this discrepancy. P2X3 glycosylation in IC was demonstrated, but the functional significance has not yet been determined. Another study used site-directed mutagenesis to determine that glycosylation of P2X3 receptor can be necessary for assembly of the functional trimeric structure and the inability to do so might result in impaired P2X3 function. The field evidently has its challenges with discrepant receptor expression between human tissues and animal models. While these initial studies indicate involvement of P2 purinergic signaling in IC, future studies are necessary to identify a suitable model and ascertain the direct functional consequence of P2 purinergic receptor upregulation.

**Urinary incontinence**

Urinary incontinence, defined as loss of bladder control or unintentional voiding, is a common condition that is often unreported. Stress incontinence results from a weak or dysfunctional urinary sphincter, urge incontinence results from overactivity of the detrusor muscle, and overflow incontinence results from bladder distention, for instance in men with BPH. Studies in rats demonstrated that activation of P2Y6 increases the voiding frequency of anesthetized rats. The authors also report that P2X3 activation is necessary for P2Y6-related increased voiding frequency.
while P2Y1 appeared to have an inhibitory effect. Furthermore, P2Y6-induced bladder hyperactivity requires intact bladder nervous circuitry since this effect was not observed in the isolated bladder in vitro. [80] Alternatively, another study found that P2Y6-deficient mice had more frequent micturition, smaller bladder capacity, and shorter bladder contraction duration than WT mice. [76] While the authors attribute this discrepancy to species differences, they also highlight that studies in rats identify P2Y6 in the bladder for its effect on voiding frequency, while the study in mice attributes the increase in voiding frequency to loss of P2Y6 in the CNS, DRG, or both. [76] In humans, P2X3 and P2X5 protein expression was notably absent in detrusors from patients with idiopathic detrusor instability when both receptors are convincingly expressed in detrusors from control patients. [81] Functional roles for P2X3 and P2X5 have not been defined in the context of urinary incontinence.

**Overactive bladder syndrome**

Overactive bladder syndrome (OAB) is characterized by urinary urgency, usually accompanied by frequency and nocturia, with or without urgency urinary incontinence, in the absence of urinary tract infection or other obvious pathology. [82] Pyuria has been associated with worst OAB symptoms. [14] One study found that there was greater ATP release from urothelium from OAB patients with pyuria than from non-OAB patients or patients with OAB but not pyuria. [14] All P2 purinergic receptors mRNA except P2X4 and P2Y4 were identified in urothelium microdissected from control patients and patients with OAB without or without pyuria (Table 1). Interestingly, compared to control patients, urothelium from OAB patients without pyuria had increased P2Y11 (200-fold) and P2Y13 (tenfold) mRNA expression, while urothelium from OAB patients with pyuria showed significantly increased P2Y2 (100-fold) and P2Y11 (50-fold) mRNA expression (n = 6; P < 0.01). [14] The suggestion is that the presence of bacteria and thereby inflammation elicits increased urothelial ATP release that plays a role in the heightened symptoms associated with pyuric OAB. Patients with OAB can have urodynamic detrusor overactivity (DO), which is the occurrence of involuntary detrusor contractions during bladder filling (phasic DO) or prior to inhibited detrusor contraction voiding at bladder capacity (terminal DO). [74, 82] P2X3 protein levels assessed by western blotting were relatively higher in DO patients than in controls (P = 0.056) and were significantly increased in the phasic DO subgroup compared to controls (n = 58, P ≤ 0.05). [74] P2X3 expression positively correlated with multiple clinical urodynamic parameters including urgency sensation and voided volume in phasic DO patients. [74] P2Y6 protein expression was more prominent in the urothelium of OAB patients compared to their age-matched male controls with BPH but no OAB symptoms. [83] P2Y6 mucosal expression positively correlated with incontinence severity (n = 28, P = 0.009), but not the International Consultation on Incontinence Questionnaire (ICIQ) OAB symptom score. [83] Both ATP and ADP, a P2Y6 agonist, were positively correlated with the ICIQ OAB symptom score, corroborating a role for purinergic signaling and likely P2 purinergic receptors in OAB. [83] P2Y12 KO mice mimic an OAB phenotype, suggestive that P2Y12 agonists might serve as a therapeutic option for OAB while P2Y12 antagonists might be useful for the treatment of bladder underactivity. [84] Further studies are needed to distinguish P2 purinergic receptor involvement in the inflammation associated with OAB and the direct effect on detrusor dysfunction.

**Bladder cancer**

Bladder cancer is the fourth most common cancer in American men and is less common in women. Fortunately, the incidence rates of bladder cancer have decreased by 1.2% per year over the last decade, but the death rate (4.4 per 100,000) has remained stable. [58] Inflammation is thought to play a role in bladder cancer development, progression, and response to treatment. [19] As previously mentioned, P2 purinergic receptors play a critical sensory role in the normal bladder, and currently relatively little is known about how these receptors act in bladder cancer. One study sought to correlate P2X receptor expression to urotherial differentiation. Western blotting and IHC analysis showed that P2X3 receptor protein expression is similar in normal urothelium (n = 13) and low-grade papillary carcinoma (n = 12), but decreased in high-grade papillary carcinoma (n = 6) of the human bladder. [85] In contrast, P2X5 receptor protein expression was present in normal urothelium and high-grade carcinoma but was diminished in low-grade carcinoma. [85] The authors propose that P2X3 correlates with urothelial differentiation and might be involved in high-grade papillary carcinoma pathogenesis, while P2X5 does not. In another study, P2Y2 enhancer RNA (P2Y2e) was overexpressed in 29 of 38 bladder cancer patient cancer tissues compared to paracancerous tissues. [86] Increased P2Y2e expression was positively correlated to high histological grade, tumor invasion, and late TNM stage. [86] Furthermore, CRISPR knockdown of P2Y2e inhibited cell proliferation, migration, and invasion and induced apoptosis in bladder cancer 5637 and T24 cells. [86] Corroborating these studies, P2Y2 receptor expression was significantly increased in urothelial carcinoma compared to normal (Fig. 3). P2Y6 receptor expression was also increased, but the majority of receptors were downregulated. P2X1, P2X2, P2X6, P2X7, P2Y1, P2Y13, and P2Y14 receptor expression was significantly decreased in tumor compared to normal in urothelial carcinoma datasets (Fig. 3).
Prostate

The prostate is the major male reproductive gland that facilitates mixing of sperm with the other components that make up semen and aid in the ejaculation of complete semen. [87] Across all species, prostate contractility is mediated by both adrenergic and purinergic receptors. [88] One proposed role for P2 purinergic receptors in normal prostate function is in P2X1-mediated contractile response of the prostate (Fig. 5). [89] In their study, White et al. demonstrate impaired nerve-mediated contractions in aged (12 months old) P2X1 knockout mice compared to aged WT mice. [89]

Another function of the prostate is likely to serve as a barrier to infection between the bladder and external environment and the remainder of the male reproductive system. [17] The urethra connects the bladder to the penis via the prostate. The testes, epididymis, seminal vesicles, and vas deferens also access the prostate for the passage of semen. Interestingly, prostate cancer is the second most common and bladder cancer the fourth most common cancer in men. [58] Meanwhile, primary cancers of the testicles, epididymis, seminal vesicle, and vas deferens are quite rare. [90–92] There is evidence that STIs contribute to prostatic inflammation and the urinary microbiome is thought to influence prostate health. [17, 93, 94] These observations likely contribute to the fact that the prostate is a site of very common diseases, all strongly associated with inflammation and also with aging. P2 purinergic receptors have been identified in prostates from rats, and this expression was reported to increase with aging (Table 1). [95] We review P2 purinergic receptor involvement in prostate diseases (Fig. 5).

Prostatitis

Prostatitis is a heterogeneous disease categorized into four syndromes: (i) acute bacterial prostatitis, (ii) chronic bacterial prostatitis, (iii) chronic prostatitis/chronic pelvic pain syndrome (inflammatory or non-inflammatory), and (iv) asymptomatic inflammatory prostatitis. [96] Although the prevalence of prostatitis is high, reported as high as 9.7% of the US population [96], the precise etiology and mechanism of disease are not always clear. Inflammation and pain are consistent characteristics in men with prostatitis. P2 purinergic receptors have been studied primarily for their nociceptive functions in prostatitis. P2X3 receptors are critical for peripheral pain responses to inflammation and tissue damage. [97] In a rat model of chemically induced prostatic inflammation, P2X3 receptor protein expression was increased in the DRG compared to control groups and a P2X3-specific antagonist attenuated ATP-induced currents in the same region. [98] The authors postulate that prostatic inflammation resulted in increased P2X3 expression, which might account for the neuronal hypersensitivity and subsequent pain associated with prostatitis. Similarly, in a mouse model of prostatitis, the percentage of thoracolumbar DRG neurons expressing P2X2 and P2X3 was significantly elevated with increasing inflammation compared to control mice (n = 4, P < 0.05). [99] In a rat model of chronic prostatitis, increased P2X7 mRNA was detected in the posterior horn of the spinal cord. [100] Inhibition of P2X7 resulted in decreased IL-1β and TNF-α production (P < 0.01) and activation of P2X7 with agonist benzoyl ATP alone was sufficient to significantly increase expression of both cytokines in the spinal cord (P < 0.01 and P < 0.05, respectively). [100] The authors propose that P2 purinergic receptor-mediated inflammation might contribute to the chronic pain associated with prostatitis.

BPH and lower urinary tract symptoms

BPH is described as the progressive enlargement of the prostate gland resulting from nonmalignant proliferation. BPH is prevalent in aging men, with prevalence rates reported from 50 to 75% among men 50 years of age and older to 80% among men 70 years of age and older. [16] White et al. proposes that a P2X1 purinergic contractile response to nerve stimulation develops in the mouse prostate gland with age.
This might contribute to the increased muscular tone observed in BPH and highlights P2X1 as a target for the treatment of BPH. [89] BPH can be asymptomatic; however, about half of the men with BPH have lower urinary tract symptoms which include a range of etiologies that are poorly understood. [16] Detrusor underactivity, which leads to incomplete bladder emptying, is one potential symptom. Several studies have identified eATP as necessary for detrusor contractility and increased eATP is associated with increased bladder sensation and voiding frequency. [69, 70, 80, 101, 102] One study identified P2Y6 as a potential channel for ATP release from mucosal urethrium. [72] A selective P2Y6 agonist significantly increased ATP release from stimulated mucosa of control bladder samples \( (P < 0.05) \) and this release was significantly attenuated \( (P < 0.05) \) by an irreversible P2Y6 antagonist. [72] The authors propose that eATP released via P2Y6 might activate P2X3 and/or P2X2/3 receptors, which in turn release more ATP creating a feedback loop. In the same study, urethrium/lamina propria (U/ LP) strips from patients with BPH \( (n=6) \) released significantly more ATP than U/LP strips from control patients \( (n=5) \) \( (P < 0.05) \). [72] Furthermore, they used high-performance liquid chromatography experiments to assess ATP catabolism and found a near doubling in the half degradation time of ATP \( (30 \mu M) \) in BPH patients \( (n=4) \) compared to control men \( (n=4) \) \( (P < 0.05) \). [72] The combined effects of increase ATP release and decreased ATP degradation might contribute to the high nucleotide levels observed in the bladder mucosa. P2Y6 was identified as the receptor responsible for this release, since P2Y6 agonist PSB0474 increased ATP release and P2Y6 antagonist MRS2578 significantly reduced ATP release in both BPH and control patients \( (P < 0.05) \). [72] Immunofluorescence confocal microscopy demonstrated a dramatic reduction in P2X2 and P2X3 protein expression in tissues with BPH compared to control tissues. [72]

**Prostate cancer**

Prostate cancer is the second leading cause of cancer-related deaths in American men. [58] Chronic inflammation might contribute to prostate carcinogenesis and is associated with aggressive prostate cancer. [17] Phagocytes, including macrophages and neutrophils, respond to inflammatory stimuli by releasing reactive oxygen and nitrogen species that can cause DNA damage, cell injury, and cell death. [17] Consequently, inflammation, cell injury, and cell death result in ATP release and increased eATP concentrations, which can activate P2 receptors on both immune and prostate cells. For instance, P2X4 receptors are involved in neutrophil recruitment, macrophage activation, and differentiation. [103, 104] P2X4, P2X5, P2X7, P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13, and P2Y14 receptor expression has been identified in prostate cancer cells (Table 2). [105–108] P2Y1 activation induces apoptosis and inhibits proliferation of PC3 cells. [105] Activation of P2X5 and/or P2Y11 is proposed to substantially inhibit growth of hormone refractory prostate cancer PC3 and DU145 cells and induce Ca \(^{2+} \)-independent apoptosis. [109] Alternatively, another study suggests that eATP stimulates DU145 cell proliferation, but the receptor subtype was not identified. [110] The authors propose that hypotonic stress results in ATP release from DU145 cells via intracellular Ca \(^{2+} \) signaling, resulting in the activation of ERK1/2, p38, and PI3K, which can facilitate cell proliferation. [110] ATP treatment of PC3 sub-clones, 1E8 (metastatic), and 2B4 (non-metastatic) cells stimulates cell invasion via the Erk1/2 and p38 pathways. [111, 112] ATP treatment alone was also shown to enhance cell motility and cell invasion of DU145, 1E8, and 2B4 cells and altered EMT-related genes, IL-8, Snail, E-cadherin, and Claudin-1. [106, 112] ATP treatment increases the number and length of lamellipodia and filopodia, which are necessary for cell motility as well as their associated Rho GTPases, Rac1 and Cdc42. [112] Furthermore, ATP induces MMP-3 or MMP-13 in DU145, 1E8, and 2B4 cells via AKT and ERK1/2 signaling. [112] P2Y2 siRNA-mediated knockdown identified P2Y2 as one receptor facilitating ATP-induced 1E8 and 2B4 cell migration and invasion. [106] Stable clones of 1E8 P2Y2 shRNA knockdown injected into nude mice did not result in liver metastasis while control cells resulted in liver metastases in 50% of mice. [106] Knockdown of P2Y2 also resulted in reduced tumor growth. [106]

P2X7 receptor protein expression was absent from normal prostate epithelium obtained at autopsy \( (n=6) \) and tissue collected from younger men by transurethral resection \( (n=17) \). [11] Conversely, P2X7 receptor protein expression was detected in 100% of confirmed prostate cancer tissues obtained by biopsy \( (n=116) \), regardless of Gleason score. [11] The authors note that P2X7 expression was detected in earlier biopsies from patients who were eventually diagnosed with prostate cancer, even though the biopsy tissues were considered normal by H&E staining. [11] The authors postulate this finding indicates that P2X7 is a potential diagnostic marker, even in cases where biopsies might initially miss the prostate cancer. In a later study, this group demonstrated that P2X7 expression correlates positively with PSA levels. [113] Specifically, biopsies that were negative for P2X7 were from patients with PSA < 2 ng/mL. [113] The P2X7 described in these studies is described as non-pore functional P2X7 (nP2X7). It is proposed that high ATP concentrations in the TME drive higher expression of nP2X7, as demonstrated in vitro. This nP2X7 was reported as necessary for PC3, DU145, and LNCaP prostate cancer cell survival. [114] Further evidence for P2X7 receptor involvement in prostate cancer is the significant association between the rs3751143 SNP in the P2X7 gene and prostate cancer (odds ratio = 0.86,
The P2X4 antagonist 5-(3-Bromophenyl)-1,3-dihydropyrimidine (5-BDBD) was shown to reduce CSM relaxation and induce IPA and vas deferens contractions in rats. [130–132] P2 receptor-mediated responses were decreased in vitro. [127–129] Similarly, P2Y1 activation induced contractions in the internal pudendal arteries (IPA), the major blood supply to the penis. [130] Meanwhile, P2X1 activation appeared to induce urinary incontinence and decrease CSM relaxation and induce IPA and vas deferens contractions in rats. [130–132] P2X4 expression was also increased, while P2X1, P2X2, P2X6, P2X7, P2Y2, P2Y13, and P2Y14 receptor expression was decreased (Fig. 3).

Future studies are necessary to characterize the expression profiles of individual receptors, determine their function, and ultimately assess the feasibility of therapeutically targeting P2 purinergic receptors in prostate cancer.

### Erectile dysfunction and infertility

Purinergic signaling is important for initiation and maintenance of penile erection. [127] As such, P2 purinergic receptors have been investigated for their role in erectile dysfunction (ED). Rabbit and human cavernosal smooth muscle (CSM) relaxation is mediated by P2Y1, P2Y2, or P2Y6 receptor activation. [127–129] Similarly, P2X4 antagonists have been shown to reduce CSM relaxation and induce IPCA and vas deferens contractions in rats. [130–132] P2X1 activation appeared to induce urinary incontinence and decrease CSM relaxation and induce IPA and vas deferens contractions in rats. [130–132] P2X4 antagonists have been shown to reduce CSM relaxation and induce IPCA and vas deferens contractions in rats. [130–132] P2X1 activation appeared to induce urinary incontinence and decrease CSM relaxation and induce IPA and vas deferens contractions in rats. [130–132]
in corpora cavernosum tissues from castrated rats and P2 purinergic receptor expression was positively correlated with serum testosterone levels. [137] Further studies are necessary to deduce whether certain pathologies dysregulate P2 purinergic expression or function to contribute to ED. To date, P2X1 antagonism has been shown to inhibit IPA or vas deferens contraction, P2Y1 antagonism has been shown to inhibit IPA relaxation in rats, and intracavernous injection of P2 purinergic agonist, suramin, improves recovery of ED in a rat model. [130, 132, 138] These data are promising for potential P2 purinergic receptor-based therapeutic options for men with ED.

P2X1-mediated vas deferens contraction has also been studied in the context of fertility. Mice lacking P2X1 have 90% reduced fertility due to reduced sperm in the ejaculate. [139] Evidence suggests that the absence of P2X1 affects sperm transport, but not sperm function in mice. [139, 140] There was also no effect on sexual behavior or function. [140] Consequently, P2X1 has been proposed as a pharmacological target for reversible male contraception, likely in concert with α1A-adrenoceptor antagonism. [140] Other P2 purinergic receptors are thought to contribute to infertility. P2X4 and/or P2X7 protein expression is observed in human testicular peritubular cells and ATP treatment of these cells resulted in pro-inflammatory cytokine production and secretion. [141] These data suggest that P2 purinergic receptors might promote sterile testicular inflammation, which is associated with infertility. ATP activation of peritubular cells via P2 purinergic receptors was shown to drive testicular sperm transport, suggesting a pharmacological target for male infertility and contraception. [142]

Ectonucleotidase control of P2 purinergic receptor agonists in urologic diseases

The regulation of P2 purinergic receptor signaling involves both the modulation of receptor expression as well as the control of agonist availability. Ectonucleotidases are key enzymes that hydrolyze nucleotides, which dictate the duration of activity of P2 purinergic receptor agonists. Of the four major ectonucleotidase families, ecto-nucleoside triphosphate diphosphohydrolases (NTPDases) and ectonucleotide pyrophosphatase/phosphodiesterases (NPPs) hydrolyze ATP to ADP and ADP to AMP. [143] The eight NTPDases are generally expressed in all tissues and NTPDase1/CD39, the best characterized ectonucleotidase, is widely expressed on immune cells. [143] Others have reviewed the roles of ectonucleotidases in inflammation, pain, and cancer. [143–146] Similarly, studies are ongoing to investigate the role of ectonucleotidases in urologic systems. Specifically, Dwyer et al. provide a comprehensive review of ectonucleotidases involved in normal kidney function and renal disease. [147] The authors discuss roles for CD39 in renal inflammation, immunomodulation, acute kidney injury, chronic kidney disease, diabetic nephropathy, polycystic kidney disease, transplantation, and renal cell carcinoma. [147] All eight NTPDases were detected in the mouse urinary bladder with different localizations. [148] Specifically, CD39 was detected primarily in endothelial cells of the detrusor and lamina propria which is consistent with its role in degrading ATP within vasculature aimed at modulating thrombotic events. [148] CD39 also tightly regulates ADP-induced P2Y12-mediated bladder smooth muscle contraction. [149] Normal vas deferens contraction is also dependent on CD39. [150] CD39 knockout male mice had reduced fertility due to reduced sperm emission. [150] The authors demonstrated in vitro that P2X1 agonist-mediated contraction was reduced in vas deferens isolated from CD39 knockout mice compared to control mice. [150]

High CD39 protein expression was detected in 43.8% of bladder cancer cases and was associated with non-muscle-invasive phenotype (P < 0.001), and lower tumor stage (P < 0.001). [151] Interestingly, data from multiple datasets via ONCOMINE show that while overexpressed in most solid tumors, CD39 mRNA expression was significantly decreased in bladder and prostate cancers. [146] Another study measured reduced CD39 protein expression on macrovesicles isolated from prostate cancer patients compared to healthy individuals. [152] However, there was increased ATP and ADP hydrolysis in prostate cancer patients compared to healthy individuals, suggesting involvement of alternative ectonucleotidases. [152] The likely candidate is the NPP family, since increased NPP activity was measured in serum and platelets from prostate cancer patients compared to healthy volunteers. [153] Interestingly, there was significantly less ATP hydrolysis in patients with clinical stage IIB and III compared to IIA, suggesting increased availability of eATP in the tumor microenvironment of later stage prostate cancer patients. [152] Altogether, these data suggest that ectonucleotidases are integral in the P2 purinergic receptor signaling associated with tumorigenesis. As such, future investigations on P2 purinergic receptors in urologic cancers must include considerations for ectonucleotidases and their control of P2 purinergic receptor agonists.

Current state of P2 receptor therapies

We have discussed critical roles for P2 purinergic receptors in urologic disorders, highlighting the receptors as potential therapeutic targets. Since 1997, P2Y12 antagonists
have been FDA-approved as antiplatelet therapies [154]. Subsequently, multiple P2 purinergic receptor agonists and antagonists have entered clinical trials for various indications, including inflammation-related disorders and pain. Specifically, previous and current clinical trials have investigated the P2X3 antagonist gefapixant in treatment of IC/BPS (NCT01569438) [155], pain (NCT01554606) [155], and stress urinary incontinence (NCT04193176) [157]. A phase 2, double-blind, placebo-controlled, randomized study to assess the efficacy of gefapixant in female subjects with moderate to severe pain associated with IC/BPS after 4 weeks of treatment was conducted. Gefapixant-treated patients (n = 36) had a decrease in Numeric Pain Rate Scale (NPRS) score, Painful Bladder/Interstitial Cystitis Symptom Diary (PBIC-SD) score, O’Leary-Sant Interstitial Cystitis Symptom Index (ICSI), and Genitourinary Pain Index (GUPI) compared to patients treated with placebo (n = 38) [155]. There were no serious adverse events and only mild adverse events, primarily dysgeusia/hypogeusia [155].

Another phase 2 placebo-controlled, randomized study was conducted to assess the efficacy of a single dose level of gefapixant in subjects with moderate to severe pain associated with osteoarthritis of the knee 4 weeks after treatment. There was also a decrease in the primary outcome measure, NRPS, in gefapixant-treated patients (n = 85) compared to placebo-treated patients (n = 86) [156]. However, there was no improvement in the secondary outcomes, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), and walking pain scores [156]. Finally, a phase 3 double-blind, placebo-controlled, randomized study to evaluate the efficacy and safety of gefapixant in improving symptoms of cough-induced stress urinary incontinence (SUI) in adult female participants with refractory or unexplained chronic cough is currently in progress [157]. These trials as well as other pre-clinical studies demonstrate that P2X3 antagonists show tremendous promise in pain management across urologic diseases [5, 97].

P2X7 antagonists are in clinical trials for inflammation-related depression (NCT04116606) [158], rheumatoid arthritis (NCT00628095 [159], NCT00520572 [160]), and inflammatory pain (NCT00849134 [161]). The phase 2 study to evaluate the antidepressor efficacy of P2X7 antagonist JNJ-54175446 was suspended due to the COVID-19 pandemic [158]. Both phase 2 studies evaluating the efficacy of P2X7 antagonists, CE-224,535 [159] and AZD9056 [160], in the treatment of patients with rheumatoid arthritis showed no significant benefit of the drug compared to placebo. Both drugs were well-tolerated by patients [159, 160]. The single-blind, placebo-controlled, randomized phase 1 study was a first-time-in-human trial for the P2X7 antagonist GSK1482160 developed to treat inflammatory pain. Ali et al. used a model-based approach to predict that adequate pharmacological engagement of P2X7 could not be achieved in vivo using safe human exposures [162]. As such, development of the drug was discontinued. The therapeutic relevance of P2X7 receptors in patients with inflammatory pain remains to be tested. New P2X7 antagonists are currently in phase 1 trials to assess safety and tolerability (NCT03151486) [163] (NCT02587819) [164] or P2X7 receptor occupancy (NCT03437590) [165] in healthy individuals, which is exciting for the prospect of P2X7 antagonist therapeutics. As we have reviewed above, there is evidence for P2X3- and P2X7-mediated inflammatory pain in prostatitis [98, 100], which provides rationale for pre-clinical studies to test the efficacy of P2X3 and P2X7 antagonists in this context. Pre-clinical studies are also warranted to investigate targeting P2X7 for the treatment of CKD.

P2X7 receptor expression profiles improved kidney cancer prognostic accuracy as described previously. [62] P2X7 expression is also detected in prostate cancer, but never in benign prostate tissues [11] and a SNP in the P2X7 gene is significantly associated with prostate cancer aggressiveness (P = 0.019). Consequently, P2X7 receptors should be investigated as potential biomarkers in these cancer types. Safety and tolerability studies have been completed for a P2X7 antagonist that has potential as a basal cell carcinoma therapeutic (NCT02587819) [164]. This anti-nf-P2X7 antibody ointment proved safe and well-tolerated, with 65% of patients showing a reduction in lesion area (n = 21) [166].

A P2Y2 agonist, diquafosol tetrasodium ophthalmic solution, has completed phase 3 clinical trials (NCT00403975 [167], NCT006000288 [168], NCT00404131 [169], NCT00037661 [170]) in the USA and is approved in Japan and Korea for the topical treatment of dry eye disease [171]. One study demonstrated that 3% diquafosol may be an effective and safe treatment for the management of dry eye resulting from cataract surgery in patients with preexisting dry eye (NCT02608489) [172]. Another P2Y2 agonist, denufosol tetrasodium, completed phase 3 clinical studies to assess its efficacy in patients with cystic fibrosis (NCT00625612) [173]. While the treatments were well-tolerated, there was no benefit to the treatment group (n = 233) compared to the placebo group (n = 233) with respect to pulmonary function or the incidence of pulmonary exacerbations [174]. As new generation agonists progress from pre-clinical studies, we look forward to studies investigating blood pressure control by managing renal Na+ excretion. Similarly, the blockade of P2X1 showed great promise as a reversible male contraceptive and treatment for ED [140]. However, P2X1 antagonists have not progressed to the clinical testing phase.
Conclusions

P2 purinergic receptors are involved in normal kidney, bladder, and prostate function. Cell injury, necrosis, or apoptosis increase eATP concentrations and, subsequently, P2 purinergic receptor expression. The consequence is receptor dysregulation that might (i) directly disrupt normal urologic functions facilitating pathogenesis, (ii) mediate inflammation associated with urologic diseases, or (iii) facilitate pain associated with urologic diseases. Perhaps all of these might occur simultaneously. While key receptors are identified in some diseases and mechanisms proposed for their function, substantial gaps of understanding remain. Thorough characterization of P2 purinergic receptor expression profiles and function is necessary to bolster the current optimism surrounding therapeutic targeting of P2 purinergic receptors in urologic disease.

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Declarations

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