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Protease-activated receptor 2: a promising therapeutic target for women's cancers

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Abstract

Cancers affecting women, such as breast, uterine, ovarian, endometrial and cervical cancers, have become increasingly prevalent. The growing incidence and death rates associated with these cancers warrant the development of innovative and alternative approaches to current treatments. This article investigates the association of women's cancers with a molecular target known as protease-activated receptor 2 (PAR2), a G-protein coupled receptor that is expressed on the surface of cancer cells. Expression levels of the PAR2 gene were curated from publicly available databases and were found to be significantly overexpressed in tissues from patients with breast, uterine, ovarian, endometrial or cervical cancer compared to normal tissues. PAR2 overexpression has been previously linked to tumor progression and, in some cases, tumor growth. Activation of PAR2 by either endogenous proteases or synthetic agonists triggers certain downstream intracellular signaling pathways that have been associated with tumor progression, cell migration and invasion, angiogenesis and apoptosis of cancer cells. While recent advances have led to the identification of several PAR2 antagonists, none has yet been developed for human use. Additionally, PAR2 inhibition has been shown also to increase the efficacy of chemotherapeutic drugs, allowing them to be potentially used at less toxic doses in combination therapies for cancer. The present work briefly summarizes the current status of PAR2 as a potential therapeutic target for treating women's cancers.

Significance Statement

This article highlights potential roles for PAR2 in cancers affecting women. Overexpression of the PAR2 gene in women's cancers is associated with various oncogenic processes such as tumor progression, cell migration and invasion, ultimately contributing to poorer patient prognoses. Given the increasing incidence of women's cancers, there is an urgent need to

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develop novel therapeutic drugs and PAR2 represents a promising target for developing new treatments.

Introduction

Cancer is the second largest cause of death, accounting for approximately 10 million deaths worldwide in 2020 (WHO, 2022). Cancer collectively describes different kinds of malignant tumors, often distinguished by which tissue is affected, and typically involves mutated or abnormal cells that grow in one tissue and spread or metastasize to other tissues. Primary tumors may be tolerated unless on a major organ, but secondary tumors produced through metastases frequently lead to death because they are more difficult to detect and treat. Cancers specifically affecting women are gynecological cancers that develop in the women's reproductive system and include uterine (endometrial), ovarian, vaginal, vulvar and cervical cancer. While breast cancer affects both men and women, only 0.5-1% of all breast cancer cases occur in men (WHO, 2022). The World Health Organization (WHO) has reported that the most commonly occurring cancer among women is breast cancer (2.3 million women diagnosed and 685,000 deaths worldwide in 2020), followed by other gynecological cancers such as cervical and ovarian (WHO, 2022). It is estimated that there will be more than 3 million new cases of breast cancer and 1 million deaths per year by the year 2040 (Arnold et al., 2022). Due to this increasing incidence and rapidly rising mortality rates, there is a global need to develop novel and effective treatments for women's cancers. Early diagnosis and increased awareness amongst women remain major challenges in reducing the cancer burden (Duffy et al., 2020).

Current treatment options for cancer include chemotherapy and surgery, depending on the size and location of the tumor, the stage of the cancer, and overall patient health (Division of Cancer Prevention and Control, 2023). Despite new advances in cancer treatment, resistance

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to chemotherapeutic drugs and unwanted side effects like high cardiotoxicity substantially impact the survival rate of cancer patients (Prihantono and Faruk, 2021). Among common chemotherapeutic drugs used to treat breast cancer are docetaxel, paclitaxel, doxorubicin and capecitabine, which can be given as a monotherapy or adjuvant therapy for early-stage breast cancer (Waks and Winer, 2019). However, patients with breast cancer treated either with anthracyclines and/or taxanes commonly gain resistance to one or both drug treatments, leaving the patient with a limited range of alternative treatment options that generally have low response rates (Rivera and Gomez, 2010). More than 30% of women diagnosed with breast cancer at an early-stage progress further to the metastatic stage of breast cancer (Rivera and Gomez, 2010). The majority of patients with ovarian cancer usually are diagnosed at an advanced stage (Torre et al., 2018) and have a 70% chance of disease recurrence (Du Bois et al., 2009). Similarly, for other gynaecological cancers, the standard treatment regime involves surgical intervention with complete removal of the affected reproductive organ. Such invasive surgeries have the potential to create permanent damage with the possibility of prolonged fertility issues (Gonçalves et al., 2022), and may also trigger development of micrometastases (Tohme et al., 2017).

To meet and overcome these challenges, there is an urgent need to identify new and therapeutically beneficial drug targets. The great majority of current pharmaceuticals (more than 30%) target G protein-coupled receptors (GPCRs), the largest protein family of cell surface signaling receptors (Chaudhary and Kim, 2021). GPCRs control a wide range of essential physiological responses, but some play crucial roles in modulating oncogenic processes (Chaudhary and Kim, 2021). This article focuses on one such GPCR as a possible drug target, namely protease-activated receptor 2 (PAR2). Here we report how PAR2 regulates oncogenic signaling associated with women's cancers (**Figure 1**), how expression

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of this gene varies in women's cancers (breast, uterine, ovarian, endometrial, cervical), how its mechanism of action and intracellular signaling are driven by endogenous proteases, and why agonists or antagonists that respectively activate or inhibit PAR2-mediated functions may impact on the progression or treatment of cancers.

PAR2 in women's cancers

PAR2 is a member of the protease-activated receptor (PAR) family (PAR1-4) that belongs to a diverse group of rhodopsin-like GPCRs, which are membrane-spanning cell surface proteins (Adams et al., 2011). PAR2 has been reported to be highly expressed in human cancers (Ungefroren et al., 2017; Jiang et al., 2018). To support an association between PAR2 and women's cancers, expression of the PAR2 gene (*F2RL1*) is analysed here from publicly available databases using the University of California Santa Cruz (UCSC) Xena platform (Goldman et al., 2020) (**Figure 2**). The UCSC Xena platform curates publicly available genomic data, including from The Cancer Genome Atlas (TCGA) Pan-Cancer and the Genotype-Tissue Expression (GTEx) database, which are the largest and most commonly used databases on cancer genomic profiles and gene expression in human tissues (Consortium, 2013).

The PAR2 gene (*F2RL1*) was overexpressed in patient tissues with breast invasive carcinoma (BRCA) compared to normal breast tissues (**Figure 2a**). This is consistent with numerous studies (Qian et al., 2018; Kim et al., 2021; Kapatia et al., 2022) reported in the last decade that show involvement of PAR2 in breast cancer. *In vitro* studies have also demonstrated that high PAR2 expression in breast tumor specimens and human cell lines (BT549, MCF-7, MDA-MB-231, MDA-MB435S, MDA-MB-436, SK-BR3 and ZR-75-1) correlates with breast cancer cell migration (Su et al., 2009). A PAR2 agonist was shown to induce

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chemokinesis in MDA-MB-231 and MDA-MB-436 breast cancer cell lines (Su et al., 2009). Another study reported higher PAR2 levels in triple negative breast cancer (TNBC) patient tissues (n = 88) compared to non-TNBC patient tissues (n = 74). PAR2 levels were also significantly elevated in the lymph node of TNBC patients suggesting PAR2 as a biomarker for TNBC and a possible therapeutic target (Kapatia et al., 2022). Hormonal influences are intricately linked in breast cancer (Mitra et al., 2022), with estrogen receptor (ER) signaling pathways being central to tumor progression, while positive ER status tumors often respond to hormonal therapies (Ali and Coombes, 2002). PAR1 and PAR2 protein expression was shown to correlate differently with breast cancer aggressiveness, depending on ER status (Lidfeldt et al., 2015). Patients with high PAR2 protein expression have a significantly higher hazard ratio = 5.5 in the ER positive group ($PAR2^{High}$ ER^{+}) compared to a hazard ratio = 1.2 in the ER negative group ($PAR2^{High}$ ER^{-}) group (Lidfeldt et al., 2015). Similarly, estrogen response element motifs were found within human *F2RL1* (PAR2) promoter, and estrogen upregulated PAR2 expression in breast cancer cells (Nag and Bar-Shavit, 2018). Cooperative signaling and/or heterodimer formation of a PAR1–PAR2 complex has previously been shown (Lin and Trejo, 2013). In breast cancer, PAR2 has been reported to drive PAR1–PAR2 induced signaling pathways, soft colony formation, invasion and progression of a xenograft model of breast cancer, but not vice versa (Jaber et al., 2014). Consistent with other studies, PAR2 is shown to be essential for PAR1-driven smooth muscle cell proliferation, neointimal hyperplasia (Sevigny et al., 2011), and fibrosis (Lin et al., 2015). Together, these findings highlight the importance of understanding crosstalk between ER status and PAR1–PAR2 signaling pathways in shaping the phenotype of breast cancer.

PAR2 has also been associated with granulocyte colony-stimulating factor 2 (CSF2), which is a cytokine known to be overexpressed in tumors. PAR2 and CSF2 were reported to be

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significantly overexpressed in metastatic breast cancer cell lines (4T1 and MDA-MB-231). PAR2 activation by a peptide agonist contributed to increase in CSF2 gene expression, resulting in breast cancer tumor progresssion (Carvalho et al., 2018). Tissue factor, an agonist of PAR2, was linked to tumor progression, and PAR2 expression were significantly correlated in patients (n=172) experiencing recurrence of breast cancer tumors. Tissue factor induced PAR2 signaling was shown to play a pivotal role in poor prognosis of patients and linked to recurrence of breast cancer (Rydén et al., 2010).

Similarly, TCGA and GTEx databases show that the PAR2 gene (*F2RL1*) was significantly overexpressed in women's gynecological cancers, such as uterine carcinosarcoma (UCS) (**Figure 2b**), ovarian cancer (OV) (**Figure 2c**), uterine corpus endometrial carcinoma (UCEC) (**Figure 2d**) and cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) (**Figure 2e**), compared to normal tissues. The distinctly higher overexpression of PAR2 in cancer tissues, relative to normal tissues, suggests the possibility of PAR2 being important in women's cancer. A study of 61 uterine endometrial cancer tissues revealed a high PAR2 histology score that correlated with high PAR2 gene expression, compared to 15 normal endometrium tissue samples (Jahan et al., 2007). PAR2 upregulation in uterine endometrial cancer tissues is indicative of PAR2's involvement in tumor progression via angiogenesis (Jahan et al., 2007). PAR2 activation has been reported to promote proliferation and inhibit apoptosis in human HeLa cervical cancer cells and in primary human cervical cells (Shanshan et al., 2019). On the other hand, a PAR2 antagonist reportedly reduced both HeLa cell growth and PAR2 protein expression in nude mice (Shanshan et al., 2019). A recent study reported a positive correlation between PAR2 expression and metastatic characteristics in 119 clinical CESC tumor samples, suggesting a

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potential role for PAR2 as a prognostic marker of metastasis in CESC patients (He et al., 2021).

PAR2 in ovarian cancer has attracted recent attention with studies showing PAR2's involvement in various oncogenic processes during tumor progression. One study showed that high PAR2 expression in 95 ovarian clear cell carcinoma tissues was associated with tumor progression and shorter survival in ovarian cancer patients (Aman et al., 2017). Another study showed that PAR2 was significantly overexpressed in human ovarian cancer tissues (n=1200) compared to normal tissues (Jiang et al., 2021a). *In vitro* studies showed high PAR2 expression in high-grade serous ovarian cancer cell line (OV90) and that PAR2 activation by the synthetic peptide agonist 2f-LIGRL-NH_2 can induce cancer cell migration and invasion of OV90 cells, which was inhibited by PAR2 antagonist (I-191) or CRISPR-Cas9 knockout of the PAR2 gene (Jiang et al., 2021a).

PAR2 and its activation mechanisms

PAR2 is generally expressed on several types of immune cells such as T-cells, neutrophils, eosinophil, monocytes, mast cells, and on numerous epithelial and endothelial cells including of the lungs, liver, heart, smooth muscles, skin, kidney, gastrointestinal tract and pancreas (Heuberger and Schuepbach, 2019). PAR2 is implicated in the modulation of physiological processes associated with immunity, tissue metabolism, gastrointestinal functions, and neuronal signaling (Peach et al., 2023). In the context of cancer, protective or damaging roles of PAR2 are dependent on the type of stimuli or agonist, tissue type, and the presence of other environmental stimuli.

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PAR2 consists of seven transmembrane (TM) helices interconnected by three extracellular loops (ECL) and three intracellular loops (ICL). PAR2 selectively recognizes endogenous proteolytic enzymes, as well as synthetic ligands, that act as agonists in promoting PAR2 coupling to intracellular G proteins and recruitment of β-arrestins to promote signal transduction and functional diversity (Ramachandran et al., 2012). PARs have a similar seven TM helix bundle structure as other GPCRs, but are unusual in being activated by proteolytic enzymes that cleave at distinct sites in the extracellular N-terminus to create a tethered ligand (TL) that folds back and self-activates the receptor (Ramachandran et al., 2012). This interaction initiates conformational changes in the receptor, which couples to different arrangements of the heterotrimeric intracellular G proteins leading to changes in their signaling activities. Proteolytic cleavage at different sites in the N-terminus of PARs leads to different active conformations of the receptor that preferentially activate specific G protein-dependent and/or independent intracellular signaling cascades.

In the case of PAR2, the N-terminus is cleaved by trypsin-like serine proteases (trypsin, chymotrypsin, tryptase, factor Xa/VII, matriptase, kallikreins (KLK 2/4/5/6/14), granzyme A), and other proteases including elastase, acrosin, thrombin and cathepsin S (Ramsay et al., 2008; Reddy et al., 2010). The TL sequence of human PAR2 is SLIGKV-, which binds to a conserved region on ECL2 (Kennedy et al., 2020). In addition to protease-mediated activation, short synthetic peptide agonists corresponding to this TL sequence can activate PAR2 without proteolytic cleavage (Ramachandran et al., 2012). However, significantly higher concentrations (mM-μM) of these synthetic peptides are required for maximal efficacy as compared to much lower concentrations (nM-pM) of endogenous proteases (Barry et al., 2006).

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PAR2 activating proteases and synthetic agonists

Both proteases and synthetic PAR2 agonists are widely used to investigate PAR2 activated signaling. Proteases were originally linked to performing essential biological functions, such as nutrient digestion, modulating protein-protein interactions and being involved in the blood-clotting and apoptosis pathway. However, proteases have also been reported to cause tumor progression mainly due to their ability to invade the extracellular matrix and facilitate tumor cell migration and invasion (Eatemadi et al., 2017). Proteases are involved in modulating nutrient and oxygen supply for tumor growth and reportedly have higher expression at an early stage of cancer development, thereby modulating many aspects of cancer progression (Eatemadi et al., 2017). **Table 1** shows some PAR2-cleaving proteases and their involvement in regulating various oncogenic processes of women's cancers.

Proteases regulate a wide range of tumor functions, but development of protease inhibitors as potential cancer treatments has been challenging. Key problems are the pleiotropic properties of each protease, metabolic instability of both proteases and endogenous inhibitors, complicated design of more stable and bioavailable synthetic inhibitors, involvement of proteases at different stages of cancer development and progression, and diverse distribution of proteases throughout the body where they are needed to carry out other physiological functions that maintain homeostasis (Eatemadi et al., 2017). Also, not all proteases activate PAR2, some inhibit through cleaving at non-canonical sites that remove the tethered sequence or prevent protease-mediated activation of PAR2.

There are various reported synthetic PAR2 agonists, the most widely studied are derivatives of the canonical human TL peptide sequence. However, the rodent TL sequence (SLIGRL-NH_2 (Nystedt et al., 1995)) is two- to five-fold more potent than the human TL sequence

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(SLIGKV-NH_2 (Huang, 2007)) in mammalian cell-based assays (Hollenberg et al., 1996; Maryanoff et al., 2001). One of the most commonly used PAR2 peptide agonists, 2-furoyl-LIGRLO-NH_2, is a modified version of SLIGRL-NH_2 that shows 10-fold or more greater agonist potency, selectivity and stability and has been widely used to activate PAR2 in cultured cells and *in vivo* models (McGuire et al., 2004; Barry et al., 2010). The advantage of such a peptide is that it is more receptor selective than proteases, which are promiscuous in their actions. The less peptidic compound GB110 is equipotent with 2-furoyl-LIGRLO-NH_2 and has comparable PAR2-activating agonist properties (Suen et al., 2012). A more potent PAR2 agonist is the synthetic ligand, AY254 (Isox-Cha-Chg-AR-NH_2), which induces MDA-MB-231 breast cancer cell migration (Yau et al., 2016) and cytokine release from colon cancer cells via ERK phosphorylation (Jiang et al., 2017).

PAR2 signaling

PAR2 can activate two independent signaling pathways: one transduced by "classical" G protein coupled signaling and the other by G-protein independent signaling (Badeanlou et al., 2011). G protein dependent signaling **(Figure 3)** involves the heterotrimeric G protein complex that is comprised of G_α subunits coupled to a combination of G_β and G_γ subunits (Heuberger and Schuepbach, 2019). Activation of a GPCR leads to changes in the conformation of the receptor, activating the G_α subunit through exchange of phosphate from guanosine diphosphate (GDP) to guanosine-5'-triphosphate (GTP), resulting in the disassociation of $G_{\beta\gamma}$ dimer that activates downstream signaling pathways via effector proteins (Heuberger and Schuepbach, 2019). In PAR2 activation, G protein dependent signaling is activated via coupling with G_α proteins subtypes namely $G_{12/13}$, G_s, G_i and $G_{q/11}$ (Katritch et al., 2013). The $G_{12/13}$ pathway activates cytoskeletal and other associated proteins via Rho guanine nucleotide exchange factor/Ras homologue gene family member A

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(RhoGEF/RhoA) cascade, which influences muscle contraction, gastrointestinal (GI) function, cancer cell migration and invasion (Katritch et al., 2013; Jiang et al., 2021a).

The G_s pathway leads to stimulation of adenylyl cyclase which increases cyclic adenosine monophosphate (cAMP) levels (Yau et al., 2013). In contrast, G_i activation leads to inhibition of adenylyl cyclase which in turn reduces cAMP and protein kinase A (PKA) levels, but is also involved in upregulating tyrosine kinase Src/mitogen-activated protein kinase/extracellular-signal regulated kinase (Src/MEK/ERK) and mitogen-activated protein kinase (MAPK) pathways (Katritch et al., 2013). The $G_{q/11}$ pathway triggers release of the secondary messengers diacyl glycerol (DAG) and inositol 1,4,5-triphosphate (IP_3) by activating phospholipase C- β (PLC- β) (Katritch et al., 2013). The main role of IP_3 is to mobilize Ca^{2+} efflux from the endoplasmic reticulum into the cytoplasm, leading to activation of protein kinase C (PKC) and phosphorylation of downstream proteins (Katritch et al., 2013; Jiang et al., 2021a). Activation of these secondary messenger pathways by G protein subunits controls many cellular processes including tumor growth, cell migration and invasion, inflammation and cytokine production (Lim et al., 2013).

G protein-independent signaling **(Figure 3)** was identified more recently. PAR2 is known to activate several G protein-independent signaling pathways by inducing recruitment of β-arrestins or scaffolding proteins that enhance PAR2 desensitization and internalization to activate distinct signaling pathways (Adams et al., 2011). In response to PAR2 agonist, β-arrestins forms a multimolecular complex in the cytosol with PAR2 and its upstream signaling module rapidly accelerates fibrosarcoma-1 (Raf-1) (Terrillon and Bouvier, 2004). This multimolecular complex is responsible for Ras-dependent activation of ERK1/2 forming the MAPK module (Raf-1, MEK1/2 and ERK1/2) (Terrillon and Bouvier, 2004). The

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activated ERKs translocate to the nucleus, where they phosphorylate transcription factors leading to gene expression and cell proliferation (Terrillon and Bouvier, 2004). β-arrestins mediated activation of transcription factors such as nuclear factor kappa B (NF-κB) leads to expression of genes involved in innate immune responses, cell proliferation and differentiation (Macfarlane et al., 2005). Hence, PAR2 activation by either G proteins or β-arrestins leads to activation of different signaling pathways such as ERK1/2, cAMP, Rho, intracellular Ca^{2+}, NF-κB and MAPK that are associated with different disease conditions (Roychoudhury et al., 2020).

Among the PAR family, PAR2 has emerged as a significant player in cancers. When activated within the tumor microenvironment, PAR2 initiates a cascade of downstream signaling events that contribute to the hallmark characteristics of cancer. Activation of PAR2 facilitates tumor progression through mechanisms such as increased cell survival, and resistance to apoptosis via MEK1/2 and PI3K pathways (Iablokov et al., 2014), thereby promoting tumor growth. Further, PAR2 activation induces cytoskeletal rearrangements, promoting cancer cell migration and invasion via RhoA (Zhu et al., 2011; Stahn et al., 2016), PKC and ERK (Hu et al., 2013) pathways in cancer cells. PAR2 activation stimulates angiogenesis by releasing vascular endothelial growth factor A via MEK-ERK signaling pathway, supporting formation of new blood vessels to sustain tumor growth (Chang et al., 2013). Moreover, PAR2 activation leads to NF-κB signaling (Johnson et al., 2016; Kawaguchi et al., 2020) and triggers release of pro-tumor mediators (Xu et al., 2015; Mussbach et al., 2016), creating a microenvironment that supports tumor progression. Importantly, PAR2 activation induces epithelial-mesenchymal transition, a critical process in metastasis (Tsai et al., 2019), while inhibition of PAR2 prevents ERK-induced epithelial-mesenchymal transition (Jiang et al., 2022).

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PAR2 antagonists

A few PAR2 synthetic antagonists have been developed to investigate the role of PAR2 in mammalian cells and in rodent models of human diseases. Among reported PAR2 antagonists are weak inhibitory peptides (e.g. FSLLRY-NH_2 (Wei et al., 2016), LSIGRL-NH_2 (Al-Ani et al., 2002)), peptidomimetics (e.g. K-14585 (Goh et al., 2009), K-12940 (Kanke et al., 2009), C391 (Boitano et al., 2015)), small molecules (e.g. GB88 and GB83 (Barry et al., 2010; Suen et al., 2012), AZ8838 (Cheng et al., 2017), AZ3451 (Huang et al., 2019), I-191 (Jiang et al., 2018), I-287 (Avet et al., 2020)) and antibodies (e.g. SAM11 (Asaduzzaman et al., 2018)). Apart from targeting the ectodomains of PAR2, cell-permeable pepducins (e.g. P2pal-18S (Asaduzzaman et al., 2015)) bind to the ICLs of PAR2 and cyclic peptides (e.g. Pc(4-4) (Kancharla et al., 2015; Nag et al., 2022)) bind to the pleckstrin homology domain within the cytoplasmic C-terminal tail of PAR2. Some of these reported antagonists have been shown to reduce PAR2 activation in a range of disease models. However, many of these antagonists show only weak inhibitory potency, and some are biased in only inhibiting one of a few PAR2 signaling pathways. Furthermore, about half of the antagonists only inhibit activation by non-proteolytic synthetic agonists and do not inhibit PAR2 activation by endogenous proteases, so they have limited value for drug development. Currently, there is no PAR2 antagonist approved for human use. AstraZeneca has taken the first PAR2 monoclonal antibody therapy MEDI0618 to Phase I clinical trials for assessment of its safety for treating chronic pain (McIntosh et al., 2020).

Evidence for PAR2 modulation in combination with chemotherapeutic drugs

PAR2 inhibition has been recently reported to modulate chemotherapeutic drug functions, and this may be possible to exploit through combination therapies for the treatment of cancers. Combination therapies offer a range of advantages such as decreasing toxic effects

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on normal cells, enhancing selectivity, reducing chemoresistance compared to a monotherapy, increasing cytotoxicity to cancer cells and improving efficacy of treatment (Bayat Mokhtari et al., 2017). In previous work, we showed that PAR2 was overexpressed in colon adenocarcinoma tissues (n=331) compared to normal colon tissues (n=308), and that PAR2 activation via an endogenous protease agonist or a synthetic peptide agonist significantly reduced doxorubicin-induced cell death in HT29 human colon cancer cells. PAR2 inhibition fully restored doxorubicin-mediated effects, suggesting PAR2 antagonism as a possible strategy for enhancing doxorubicin chemotherapy with fewer toxic side effects (Shah et al., 2023).

Another study reported PAR2 inhibition or PAR2 depletion causes colorectal cancer cell migration suppression and reduction in epithelial-to-mesenchymal transition signaling sensitizing cells to 5-fluorouracil chemotherapy treatment. PAR2 activation also causes decrease in apoptosis of colorectal cancer cells suggesting a possible new strategy for improving 5-fluorouracil resistance and improving therapy performance in colon cancer patients (Quan et al., 2019). PAR2 has also been reported to cause resistance to chemotherapy drug gefitinib in NSCLC cells. PAR2 was reported to be expressed on NSCLC cells or tissues after gefitinib resistance. Combination of P2pal-18S PAR2 antagonist and gefitinib were responsible in blocking ERK phosphorylation in NSCLC cells. The resistance to gefitinib was reversed via inhibition of PAR2 by β-arrestin-EGFR-ERK signaling pathway. (Jiang et al., 2021b) This promise of PAR2 modulation in the treatment of cancer remains to be demonstrated for specific women's cancers.

Conclusions and future perspectives

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Since the discovery of PARs in the 1990's, there have been a number of reports of their prominent roles in various types of cancers. Multiple studies have indicated overexpression of the PAR2 gene, including in cancer tissues of women, and association with cancer progression. Publicly available databases show significant upregulation of the PAR2 gene (*F2RL1*) in breast cancer patient tissues compared to normal mammary tissues, with high PAR2 expression being linked to poor prognosis in patients. PAR2 gene expression was also found to be significantly overexpressed in uterine, ovarian, endometrial and cervical cancer tissues of female cancer patients compared to corresponding normal tissues in healthy women. This clinical data for high PAR2 expression in female cancer patients has been linked to poor prognosis, cancer cell migration and invasion, angiogenesis and tumor progression. Further research is needed to better document and correlate PAR2 expression with cancer development and progression before it can be concluded that PAR2 is a reliable biomarker for diagnosis of women's cancers.

A greater understanding of how PAR2 modulates oncogenic processes in women can be of potential benefit in developing potent new drugs with a higher safety profile, minimal side effects and anti-cancer therapeutic efficacy. This information can elaborate links between PAR2, immunometabolism and cancer biology, and point to the best PAR2-mediated strategies for treating women's cancers. It is well established that PAR2 is activated by many different endogenous but pleiotropic proteases, which in their own right have been linked to cancers. To date there are no known endogenous agonists other than proteases, but synthetic peptide and nonpeptide exogenous agonists have been developed as more selective PAR2-activating tools to better understand its activation mechanisms and signaling outcomes. PAR2 activation by proteases involves a unique mechanism in which the extracellular N-terminus of PAR2 is pruned to unmask a unique ligand sequence that folds back to self-activate the

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receptor. This activates coupling to intracellular G proteins and recruitment of β-arrestins, leading to activation of multiple downstream signaling pathways and stimulation of, for example, RhoA, MEK-ERK, PKC or Ca^{2+} signaling. These signaling pathways regulate a wide range of cellular functions ranging from metabolism, obesity, motility, infection and inflammation to tumor progression, but the latter assumes great importance due to high expression of PAR2 in and on cancer cells. A few PAR2 antagonists have been identified and shown to inhibit the activation of PAR2-mediated intracellular signaling and downstream cellular, physiological and pathophysiological responses. There is current promise for PAR2 antagonists and antibodies as new treatments for women's cancers, and for combination therapies with existing chemotherapeutic drugs with some evidence that PAR2 antagonism may reduce the cardiotoxicity and side effects of chemotherapies. PAR2 antagonism thus promises a new approach to modulate certain cancer signaling pathways, thereby providing new opportunities for the treatment of women's cancers.

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Data availability

No new data were generated for this article. Data that support the findings of this study are openly available in The Cancer Genome Atlas (TCGA) Pan-Cancer and The Genotype-Tissue Expression (GTEx) databases.

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Author contributions

Wrote or contributed to the writing of the manuscript: Himani Shah, David P. Fairlie, Junxian Lim

Abbreviations

PAR2- Protease-activated receptor 2

GPCR- G protein-coupled receptor

UCSC- University of California Santa Cruz

TCGA- The cancer genome atlas

GTEx- Genotype-tissue Expression

BRCA - Breast invasive carcinoma

UCS- Uterine carcinosarcoma

OV- Ovarian cancer

UCEC- Uterine corpus endometrial carcinoma

CESC- Cervical squamous cell carcinoma and endocervical adenocarcinoma

TNBC - Triple negative breast cancer

ER - Estrogen receptor

CSF2 - Granulocyte colony-stimulating factor 2

TM- Transmembrane

ECL- Extracellular loop

ICL - Intracellular loops

TL- Tethered ligand

GDP- Guanosine diphosphate

GTP- Guanosine-5'-triphosphate

cAMP- Cyclic adenosine monophosphate

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PKA - Protein kinase A

ERK- Extracellular-signal regulated kinase

MAPK- Mitogen-activated protein kinase

DAG- Diacyl glycerol

IP_3- Inositol 1,4,5-triphosphate

PLC- β- Phospholipase C- β

PKC- Protein kinase C

NF-κB- Nuclear factor kappa B

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References

Adams MN, Ramachandran R, Yau MK, Suen JY, Fairlie DP, Hollenberg MD and Hooper JD (2011) Structure, function and pathophysiology of protease activated receptors. *Pharmacol Ther* **130**:248-282.

Al-Ani B, Saifeddine M, Wijesuriya SJ and Hollenberg MD (2002) Modified proteinase-activated receptor-1 and-2 derived peptides inhibit proteinase-activated receptor-2 activation by trypsin. *J Pharmacol Exp Ther* **300**:702-708.

Ali S and Coombes RC (2002) Endocrine-responsive breast cancer and strategies for combating resistance. *Nat Rev Cancer* **2**:101-112.

Albrektsen T, Sorensen BB, Hjorto GM, Fleckner J, Rao LV and Petersen LC (2007) Transcriptional program induced by factor VIIa-tissue factor, PAR1 and PAR2 in MDA-MB-231 cells. *J Thromb Haemost* **5**:1588-1597.

Aman M, Ohishi Y, Imamura H, Shinozaki T, Yasutake N, Kato K and Oda Y (2017) Expression of protease-activated receptor-2 (PAR-2) is related to advanced clinical stage and adverse prognosis in ovarian clear cell carcinoma. *Hum Pathol* **64**:156-163.

Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, Vignat J, Gralow JR, Cardoso F, Siesling S and Soerjomataram I (2022) Current and future burden of breast cancer: Global statistics for 2020 and 2040. *Breast* **66**:15-23.

Asaduzzaman M, Davidson C, Nahirney D, Fiteih Y, Puttagunta L and Vliagoftis H (2018) Proteinase-activated receptor-2 blockade inhibits changes seen in a chronic murine asthma model. *Allergy* **73**:416-420.

Asaduzzaman M, Nadeem A, Arizmendi N, Davidson C, Nichols HL, Abel M, Ionescu LI, Puttagunta L, Thebaud B, Gordon J, DeFea K, Hollenberg MD and Vliagoftis H (2015) Functional inhibition of PAR2 alleviates allergen-induced airway hyperresponsiveness and inflammation. *Clin Exp Allergy* **45**:1844-1855.

Avet C, Sturino C, Grastilleur S, Gouill CL, Semache M, Gross F, Gendron L, Bennani Y, Mancini JA, Sayegh CE and Bouvier M (2020) The PAR2 inhibitor I-287 selectively targets Galpha(q) and Galpha(12/13) signaling and has anti-inflammatory effects. *Commun Biol* **3**:719.

Badeanlou L, Furlan-Freguia C, Yang G, Ruf W and Samad F (2011) Tissue factor-protease-activated receptor 2 signaling promotes diet-induced obesity and adipose inflammation. *Nat Med* **17**:1490-1497.

Barry GD, Le GT and Fairlie DP (2006) Agonists and antagonists of protease activated receptors (PARs). *Curr Med Chem* **13**:243-265.

Barry GD, Suen JY, Le GT, Cotterell A, Reid RC and Fairlie DP (2010) Novel agonists and antagonists for human protease activated receptor 2. *J Med Chem* **53**:7428-7440.

Bayat Mokhtari R, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B and Yeger H (2017) Combination therapy in combating cancer. *Oncotarget* **8**:38022-38043.

Boitano S, Hoffman J, Flynn AN, Asiedu MN, Tillu DV, Zhang Z, Sherwood CL, Rivas CM, DeFea KA and Vagner J (2015) The novel PAR2 ligand C391 blocks multiple PAR2 signalling pathways in vitro and in vivo. *Br J Pharmacol* **172**:4535-4545.

Carvalho É, Hugo de Almeida V, Rondon AMR, Possik PA, Viola JPB and Monteiro RQ (2018) Protease-activated receptor 2 (PAR2) upregulates granulocyte colony stimulating factor (G-CSF) expression in breast cancer cells. *Biochem Biophysl Res Commun* **504**:270-276.

Chang LH, Pan SL, Lai CY, Tsai AC and Teng CM (2013) Activated PAR-2 regulates pancreatic cancer progression through ILK/HIF-alpha-induced TGF-alpha expression and MEK/VEGF-A-mediated angiogenesis. *Am J Pathol* **183**:566-575.

Chaudhary PK and Kim S (2021) An insight into GPCR and G-Proteins as cancer drivers. *Cells* **10**.

Cheng RK, Fiez-Vandal C, Schlenker O, Edman K, Aggeler B, Brown DG, Brown GA, Cooke RM, Dumelin CE, Doré AS, Geschwindner S, Grebner C, Hermansson NO, Jazayeri A, Johansson P, Leong L, Prihandoko R, Rappas M, Soutter H, Snijder A, Sundstrom L, Tehan B, Thornton P, Torast D, Wiggin G, Zhukov A, Marshall FH and Dekker N (2017) Structural insight into allosteric modulation of protease-activated receptor 2. *Nature* **545**:112-115.

Consortium GTEx (2013) The Genotype-Tissue Expression (GTEx) project. *Nat Genet* **45**:580-585.

Division of Cancer Prevention and Control, Centers for Disease Control and Prevention (2023) Cancer Treatments. Accessed on January 2024. Available from: https://www.cdc.gov/cancer/survivors/patients/treatments.htm

Du Bois A, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I and Pfisterer J (2009) Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). *Cancer* **115**:1234-1244.

Duffy SW, Tabár L, Yen AM, Dean PB, Smith RA, Jonsson H, Törnberg S, Chen SL, Chiu SY, Fann JC, Ku MM, Wu WY, Hsu CY, Chen YC, Svane G, Azavedo E, Grundström H, Sundén P, Leifland K, Frodis E, Ramos J, Epstein B, Åkerlund A, Sundbom A, Bordás P, Wallin H, Starck L, Björkgren A, Carlson S, Fredriksson I, Ahlgren J, Öhman D, Holmberg L and Chen TH (2020) Mammography screening reduces rates of advanced and fatal breast cancers: Results in 549,091 women. *Cancer* **126**:2971-2979.

Eatemadi A, Aiyelabegan HT, Negahdari B, Mazlomi MA, Daraee H, Daraee N, Eatemadi R and Sadroddiny E (2017) Role of protease and protease inhibitors in cancer pathogenesis and treatment. *Biomed Pharmacother* **86**:221-231.

Goh FG, Ng PY, Nilsson M, Kanke T and Plevin R (2009) Dual effect of the novel peptide antagonist K-14585 on proteinase-activated receptor-2-mediated signalling. *Br J Pharmacol* **158**:1695-1704.

Goldman MJ, Craft B, Hastie M, Repecka K, McDade F, Kamath A, Banerjee A, Luo Y, Rogers D, Brooks AN, Zhu J and Haussler D (2020) Visualizing and interpreting cancer genomics data via the Xena platform. *Nat Biotechnol* **38**:675-678.

Gonçalves V, Ferreira PL, Saleh M, Tamargo C and Quinn GP (2022) Perspectives of young women with gynecologic cancers on fertility and fertility preservation: a systematic review. *Oncologist* **27**:e251-e264.

He S, Xu M, Xiong Z, Hu Y, Huo Q, Lu J, Lin Y and Yang L (2021) Predictive value of protease-activated receptor-2 (PAR2) in cervical cancer metastasis. *J Cell Mol Med* **25**:1415-1424.

Heuberger DM and Schuepbach RA (2019) Protease-activated receptors (PARs): mechanisms of action and potential therapeutic modulators in PAR-driven inflammatory diseases. *Thromb J* **17**:4.

Hollenberg MD, Saifeddine M and al-Ani B (1996) Proteinase-activated receptor-2 in rat aorta: structural requirements for agonist activity of receptor-activating peptides. *Mol Pharmacol* **49**:229-233.

Hu L, Xia L, Zhou H, Wu B, Mu Y, Wu Y and Yan J (2013) TF/FVIIa/PAR2 promotes cell proliferation and migration via PKCalpha and ERK-dependent c-Jun/AP-1 pathway in colon cancer cell line SW620. *Tumour Biol* **34**:2573-2581.

Huang SC (2007) Protease-activated receptor-1 (PAR1) and PAR2 but not PAR4 mediate relaxations in lower esophageal sphincter. *Regul Pept* **142**:37-43.

Huang X, Ni B, Xi Y, Chu X, Zhang R and You H (2019) Protease-activated receptor 2 (PAR-2) antagonist AZ3451 as a novel therapeutic agent for osteoarthritis. *Aging (Albany NY)* **11**:12532-12545.

Iablokov V, Hirota CL, Peplowski MA, Ramachandran R, Mihara K, Hollenberg MD and MacNaughton WK (2014) Proteinase-activated receptor 2 (PAR2) decreases apoptosis in colonic epithelial cells. *J Biol Chem* **289**:34366-34377.

Jaber M, Maoz M, Kancharla A, Agranovich D, Peretz T, Grisaru-Granovsky S, Uziely B and Bar-Shavit R (2014) Protease-activated-receptor-2 affects protease-activated-receptor-1-driven breast cancer. *Cell Mol Life Sci* **71**:2517-2533.

Jahan I, Fujimoto J, Alam SM, Sato E, Sakaguchi H and Tamaya T (2007) Expression of protease activated receptor-2 related to angiogenesis in tumor advancement of uterine endometrial cancers. *Oncol Rep* **17**:345-350.

Jiang Y, Lim J, Wu KC, Xu W, Suen JY and Fairlie DP (2021a) PAR2 induces ovarian cancer cell motility by merging three signalling pathways to transactivate EGFR. *Br J Pharmacol* **178**:913-932.

Jiang Y, Yau MK, Lim J, Wu KC, Xu W, Suen JY and Fairlie DP (2018) A potent antagonist of protease-activated receptor 2 that inhibits multiple signaling functions in human cancer cells. *J Pharmacol Exp Ther* **364**:246-257.

Jiang Y, Yau MK, Kok WM, Lim J, Wu KC, Liu L, Hill TA, Suen JY and Fairlie DP (2017) Biased signaling by agonists of protease activated receptor 2. *ACS Chem Biol* **12**:1217-1226.

Jiang Y, Zhuo X, Fu X, Wu Y and Mao C (2021b) Targeting PAR2 overcomes gefitinib resistance in non-small-cell lung cancer cells through inhibition of EGFR transactivation. *Front Pharmacol* **12**:625289.

Jiang Y, Zhuo X, Wu Y, Fu X and Mao C (2022) PAR2 blockade reverses osimertinib resistance in non-small-cell lung cancer cells via attenuating ERK-mediated EMT and PD-L1 expression. *Biochim Biophys Acta Mol Cell Res* **1869**:119144.

Johnson JJ, Miller DL, Jiang R, Liu Y, Shi Z, Tarwater L, Williams R, Balsara R, Sauter ER and Stack MS (2016) Protease-activated Receptor-2 (PAR-2)-mediated Nf-kappaB Activation Suppresses Inflammation-associated Tumor Suppressor MicroRNAs in Oral Squamous Cell Carcinoma. *J Biol Chem* **291**:6936-6945.

Kancharla A, Maoz M, Jaber M, Agranovich D, Peretz T, Grisaru-Granovsky S, Uziely B and Bar-Shavit R (2015) PH motifs in PAR1&2 endow breast cancer growth. *Nat Commun* **6**:8853.

Kanke T, Kabeya M, Kubo S, Kondo S, Yasuoka K, Tagashira J, Ishiwata H, Saka M, Furuyama T, Nishiyama T, Doi T, Hattori Y, Kawabata A, Cunningham MR, Plevin R (2009) Novel antagonists for proteinase-activated receptor 2: inhibition of cellular and vascular responses in vitro and in vivo. *Br J Pharmacol* **158**:361-371.

Kapatia G, Kaur S, Kumar S, Laroiya I, Singh G, Sharma M, Bal A and Luthra-Guptasarma M (2022) Expression of proteinase-activated receptor 2 (PAR2) as a correlate of concern in triple-negative breast cancer (TNBC). *Appl Immunohistochem Mol Morphol* **30**:446-452.

Katritch V, Cherezov V and Stevens RC (2013) Structure-function of the G protein–coupled receptor superfamily. *Annu Rev Pharmacol Toxicol* **53**:531-556.

Kawaguchi M, Yamamoto K, Kataoka H, Izumi A, Yamashita F, Kiwaki T, Nishida T, Camerer E and Fukushima T (2020) Protease-activated receptor-2 accelerates intestinal tumor formation through activation of nuclear factor-kappaB signaling and tumor angiogenesis in Apc(Min/+) mice. *Cancer Sci* **111**:1193-1202.

Kennedy AJ, Sundstrom L, Geschwindner S, Poon EKY, Jiang Y, Chen R, Cooke R, Johnstone S, Madin A, Lim J, Liu Q, Lohman RJ, Nordqvist A, Friden-Saxin M, Yang W, Brown DG, Fairlie DP and Dekker N (2020) Protease-activated receptor-2 ligands reveal orthosteric and allosteric mechanisms of receptor inhibition. *Commun Biol* **3**:782.

Kim JM, Park J, Noh EM, Song HK, Kang SY, Jung SH, Kim JS, Youn HJ and Lee YR (2021) Downregulation of matriptase suppresses the PAR-2/PLCγ2/PKC-mediated invasion and migration abilities of MCF-7 breast cancer cells. *Oncol Rep* **46**.

Kim KK, Turner R, Khazan N, Kodza A, Jones A, Singh RK and Moore RG (2020) Role of trypsin and protease-activated receptor-2 in ovarian cancer. *PLoS One* **15**:e0232253.

Lidfeldt J, Bendahl PO, Forsare C, Malmstrom P, Ferno M and Belting M (2015) Protease activated receptors 1 and 2 correlate differently with breast cancer aggressiveness depending on tumor ER status. *PLoS One* **10**:e0134932.

Lim J, Iyer A, Liu L, Suen JY, Lohman RJ, Seow V, Yau MK, Brown L and Fairlie DP (2013) Diet-induced obesity, adipose inflammation, and metabolic dysfunction correlating with PAR2 expression are attenuated by PAR2 antagonism. *FASEB J* **27**:4757-4767.

Lin C, von der Thusen J, Daalhuisen J, ten Brink M, Crestani B, van der Poll T, Borensztajn K and Spek CA (2015) Protease-activated receptor (PAR)-2 is required for PAR-1 signalling in pulmonary fibrosis. *J Cell Mol Med* **19**:1346-1356.

Lin H and Trejo J (2013) Transactivation of the PAR1-PAR2 heterodimer by thrombin elicits beta-arrestin-mediated endosomal signaling. *J Biol Chem* **288**:11203-11215.

Macfarlane SR, Sloss CM, Cameron P, Kanke T, McKenzie RC and Plevin R (2005) The role of intracellular Ca2+ in the regulation of proteinase-activated receptor-2 mediated nuclear factor kappa B signalling in keratinocytes. *Br J Pharmacol* **145**:535-544.

Maryanoff BE, Santulli RJ, McComsey DF, Hoekstra WJ, Hoey K, Smith CE, Addo M, Darrow AL and Andrade-Gordon P (2001) Protease-activated receptor-2 (PAR-2): structure-function study of receptor activation by diverse peptides related to tethered-ligand epitopes. *Arch Biochem Biophys* **386**:195-204.

McGuire JJ, Saifeddine M, Triggle CR, Sun K and Hollenberg MD (2004) 2-furoyl-LIGRLO-amide: a potent and selective proteinase-activated receptor 2 agonist. *J Pharmacol Exp Ther* **309**:1124-1131.

McIntosh KA, Cunningham MR, Bushell T and Plevin R (2020) The development of proteinase-activated receptor-2 modulators and the challenges involved. *Biochem Soc Trans* **48**:2525-2537.

Mitra S, Lami MS, Ghosh A, Das R, Tallei TE, Fatimawali, Islam F, Dhama K, Begum MY, Aldahish A, Chidambaram K and Emran TB (2022) Hormonal therapy for gynecological cancers: How far has science progressed toward clinical applications? *Cancers (Basel)* **14**.

Mussbach F, Ungefroren H, Gunther B, Katenkamp K, Henklein P, Westermann M, Settmacher U, Lenk L, Sebens S, Muller JP, Bohmer FD and Kaufmann R (2016) Proteinase-activated receptor 2 (PAR2) in hepatic stellate cells - evidence for a role in hepatocellular carcinoma growth in vivo. *Mol Cancer* **15**:54.

Nag JK and Bar-Shavit R (2018) Transcriptional Landscape of PARs in Epithelial Malignancies. *Int J Mol Sci* **19**.

Nag JK, Malka H, Sedley S, Appasamy P, Rudina T, Levi T, Hoffman A, Gilon C, Uziely B and Bar-Shavit R (2022) PH-binding motif in PAR4 oncogene: From molecular mechanism to drug design. *Mol Cancer Ther* **21**:1415-1429.

Nystedt S, Emilsson K, Larsson AK, Strömbeck B and Sundelin J (1995) Molecular cloning and functional expression of the gene encoding the human proteinase-activated receptor 2. *Eur J Biochem* **232**:84-89.

Peach CJ, Edington-MitchellLE, Bunnett NW and Schmidt BL (2023) Protease-activated receptors in health and disease. *Physiol Rev* **103**: 717-785.

Prihantono and Faruk M (2021) Breast cancer resistance to chemotherapy: When should we suspect it and how can we prevent it? *Ann Med Surg (Lond)* **70**:102793.

Qian N, Li X, Wang X, Wu C, Yin L and Zhi X (2018) Tryptase promotes breast cancer angiogenesis through PAR-2 mediated endothelial progenitor cell activation. *Oncol Lett* **16**:1513-1520.

Quan Q, Zhong F, Wang X, Chen K and Guo L (2019) PAR2 inhibition enhanced the sensitivity of colorectal cancer cells to 5-FU and reduced EMT signaling. *Oncol Res* **27**:779-788.

Ramachandran R, Noorbakhsh F, Defea K and Hollenberg MD (2012) Targeting proteinase-activated receptors: therapeutic potential and challenges. *Nat Rev Drug Discov* **11**:69-86.

Ramsay AJ, Dong Y, Hunt ML, Linn M, Samaratunga H, Clements JA and Hooper JD (2008) Kallikrein-related peptidase 4 (KLK4) initiates intracellular signaling via protease-activated receptors (PARs). KLK4 and PAR-2 are co-expressed during prostate cancer progression. *J Biol Chem* **283**:12293-12304.

Reddy VB, Shimada SG, Sikand P, Lamotte RH and Lerner EA (2010) Cathepsin S elicits itch and signals via protease-activated receptors. *J Invest Dermatol* **130**:1468-1470.

Rivera E and Gomez H (2010) Chemotherapy resistance in metastatic breast cancer: the evolving role of ixabepilone. *Breast Cancer Res* **12**:S2.

Roychoudhury S, Kumar A, Bhatkar D and Sharma NK (2020) Molecular avenues in targeted doxorubicin cancer therapy. *Future Oncol* **16**:687-700.

Rydén L, Grabau D, Schaffner F, Jönsson PE, Ruf W and Belting M (2010) Evidence for tissue factor phosphorylation and its correlation with protease-activated receptor expression and the prognosis of primary breast cancer. *Intl J Cancer* **126**:2330-2340.

Sánchez-Hernández PE, Ramirez-Dueñas MG, Albarran-Somoza B, García-Iglesias T, del Toro-Arreola A, Franco-Topete R and Daneri-Navarro A (2008) Protease-activated receptor-2 (PAR-2) in cervical cancer proliferation. *Gynecol Oncol* **108**:19-26.

Sevigny LM, Austin KM, Zhang P, Kasuda S, Koukos G, Sharifi S, Covic L and Kuliopulos A (2011) Protease-activated receptor-2 modulates protease-activated receptor-1-driven neointimal hyperplasia. *Arterioscler Thromb Vasc Biol* **31**:e100-106.

Shah H, Hill TA, Lim J and Fairlie DP (2023) Protease-activated receptor 2 attenuates doxorubicin-induced apoptosis in colon cancer cells. *J Cell Commun Signal* **17**:1293-1307.

Shanshan H, Lan X, Xia L, Huang W, Meifang Z and Ling Y (2019) Inhibition of protease-activated receptor-2 induces apoptosis in cervical cancer by inhibiting signal transducer and activator of transcription-3 signaling. *J Int Med Res* **47**:1330-1338.

Stahn S, Thelen L, Albrecht IM, Bitzer J, Henkel T and Teusch NE (2016) Teleocidin A2 inhibits human proteinase-activated receptor 2 signaling in tumor cells. *Pharmacol Res Perspect* **4**:e00230.

Su S, Li Y, Luo Y, Sheng Y, Su Y, Padia RN, Pan ZK, Dong Z and Huang S (2009) Proteinase-activated receptor 2 expression in breast cancer and its role in breast cancer cell migration. *Oncogene* **28**:3047-3057.

Suen J, Barry GD, Lohman RJ, Halili MA, Cotterell AJ, Le GT and Fairlie DP (2012) Modulating human proteinase activated receptor 2 with a novel antagonist (GB88) and agonist (GB110). *Br J Pharmacol* **165**:1413-1423.

Terrillon S and Bouvier M (2004) Receptor activity-independent recruitment of βarrestin2 reveals specific signalling modes. *EMBO J* **23**:3950-3961.

Tohme S, Simmons RL, Tsung A (2017) Surgery for Cancer: A Trigger for Metastases. *Cancer Res* **77**:1548-1552.

Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, Gaudet MM, Jemal A and Siegel RL (2018) Ovarian cancer statistics, 2018. *CA Cancer J Clin* **68**:284-296.

Tsai CC, Chou YT and Fu HW (2019) Protease-activated receptor 2 induces migration and promotes Slug-mediated epithelial-mesenchymal transition in lung adenocarcinoma cells. *Biochim Biophys Acta Mol Cell Res* **1866**:486-503.

Ungefroren H, Witte D, Rauch BH, Settmacher U, Lehnert H, Gieseler F and Kaufmann R (2017) Proteinase-activated receptor 2 may drive cancer progression by facilitating TGF-beta signaling. *Int J Mol Sci* **18**.

Waks AG and Winer EP (2019) Breast cancer treatment: a review. *JAMA* **321**:288-300.

Wei H, Wei Y, Tian F, Niu T and Yi G (2016) Blocking proteinase-activated receptor 2 alleviated neuropathic pain evoked by spinal cord injury. *Physiol Res* **65**:145-153.

World Health Organization WHO (2022) Cancer. Accessed on January 2024. Available from: https://www.who.int/health-topics/cancer

Xu W, Lim J, Goh CY, Suen JY, Jiang Y, Yau MK, Wu KC, Liu L and Fairlie DP (2015) Repurposing registered drugs as antagonists for protease-activated receptor 2. *J Chem Inf Model* **55**:2079-2084.

Yau MK, Liu L and Fairlie DP (2013) Toward drugs for protease-activated receptor 2 (PAR2). *J Med Chem* **56**:7477-7497.

Yau MK, Suen JY, Xu W, Lim J, Liu L, Adams MN, He Y, Hooper JD, Reid RC and Fairlie DP (2016) Potent small agonists of protease-activated receptor 2. *ACS Med Chem Lett* **7**:105-110.

Zhu T, Mancini JA, Sapieha P, Yang C, Joyal JS, Honore JC, Leduc M, Zaniolo K, Hardy P, Shao Z, Fan L, Hou X, Rivard GE and Chemtob S (2011) Cortactin activation by FVIIa/tissue factor and PAR2 promotes endothelial cell migration. *Am J Physiol Regul Integr Comp Physiol* **300**:R577-585.

Footnotes

No author has any conflict of interest with the contents of this article.

Figure Legends

Figure 1 - PAR2 regulates various oncogenic processes in women's cancers.
PAR2 over expression and activation in women's cancers causes apoptosis, cancer cell proliferation, metastasis, angiogenesis, invasion, cytokine production, recurrence of cancer and poor prognosis in patients. Figure created with Biorender.com.

Figure 2 - PAR2 gene expression in women's cancers.
PAR2 (*F2RL1*) expression (log_2) between normal and cancer tissue from TCGA and GTEx databases (January 2024) in **a-** breast invasive carcinoma (BRCA) (normal n=290, cancer n=1098), **b-** uterine carcinosarcoma (UCS) (normal n=78, cancer n=57), **c-** ovarian cancer (OV) (normal n=88, cancer n=426), **d-** uterine corpus endometrial carcinoma (UCEC) (normal n=23, cancer n=180) and **e-** cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) (normal n=13, cancer n=306). All human patient data was obtained

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via UCSC Xena server and expressed as a scatter plot with means indicated by red lines. P value was calculated using Mann-Whitney test, **** $p < 0.0001$.

Figure 3 - Schematic of PAR2 mediated signaling.
PAR2 activation can lead to coupling of G protein subunits: $G_{12/13}$, G_s, G_i and $G_{q/11}$. $G_{12/13}$ can activate RhoA via RhoGEFs. G_i inhibits adenylate cyclase causing inhibition of cAMP, G_s coupling leads to increase in cAMP accumulation, $G_{q/11}$ induces activation of PLC- β which activates IP3 and DAG downstream resulting in Ca^{2+} release and activation of PKC. Coupling via β-arrestins leads to activation of MEK-ERK signaling pathway. All the various signaling mechanism, post PAR2 activation, can modulate a range of biological functions such as inflammation, tumor progression, cell migration and invasion and gene expression. Figure created with Biorender.com.

Table

Table 1 - Some PAR2-cleaving proteases involved in women's cancers.

PAR2-cleaving protease	Significance in women's cancers
Factor Xa/VIIa	- FVIIa causes transcription of various genes involved in breast cancer metastasis and angiogenesis (Albrektsen et al., 2007).
Mast cell tryptase	-Tryptase promotes breast cancer cell angiogenesis via PAR2 mediated activation of ERK and Akt signaling pathways (Qian et al., 2018).
Matriptase	- Matriptase induces breast cancer cell migration and invasion in MCF-7 cells (Kim et al., 2021).
Trypsin	-Trypsin induces tumor proliferation in cervical cancer cell lines (Sánchez-Hernández et al., 2008). -Trypsin causes MAPK signaling and increased proliferation of ovarian cancer cell lines (Kim et al., 2020). -Trypsin induces migration and invasion of OV90 ovarian cancer cell line through activating $G\alpha_{q/11}$, $G\alpha_{12/13}$ and β-

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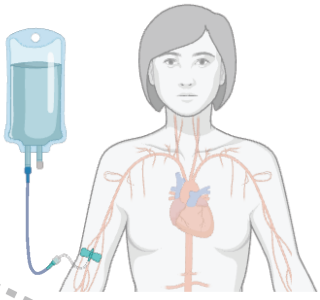
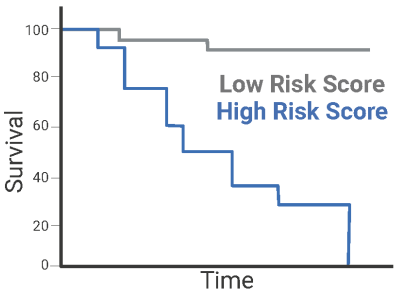
	arrestin1/2 signaling pathway (Jiang et al., 2021a).

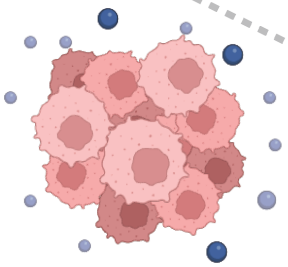
Figure 1



Cancer recurrence

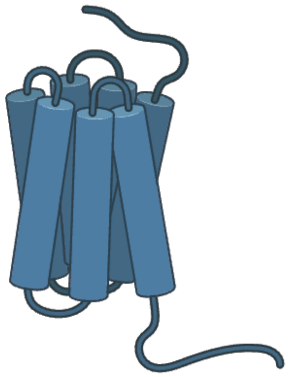


Poor prognosis

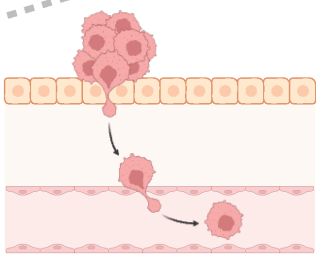


Cytokine production

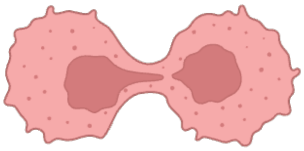
Apoptosis



PAR2 in women's cancer



Invasion



Proliferation

Angiogenesis

Metastasis

Figure 2

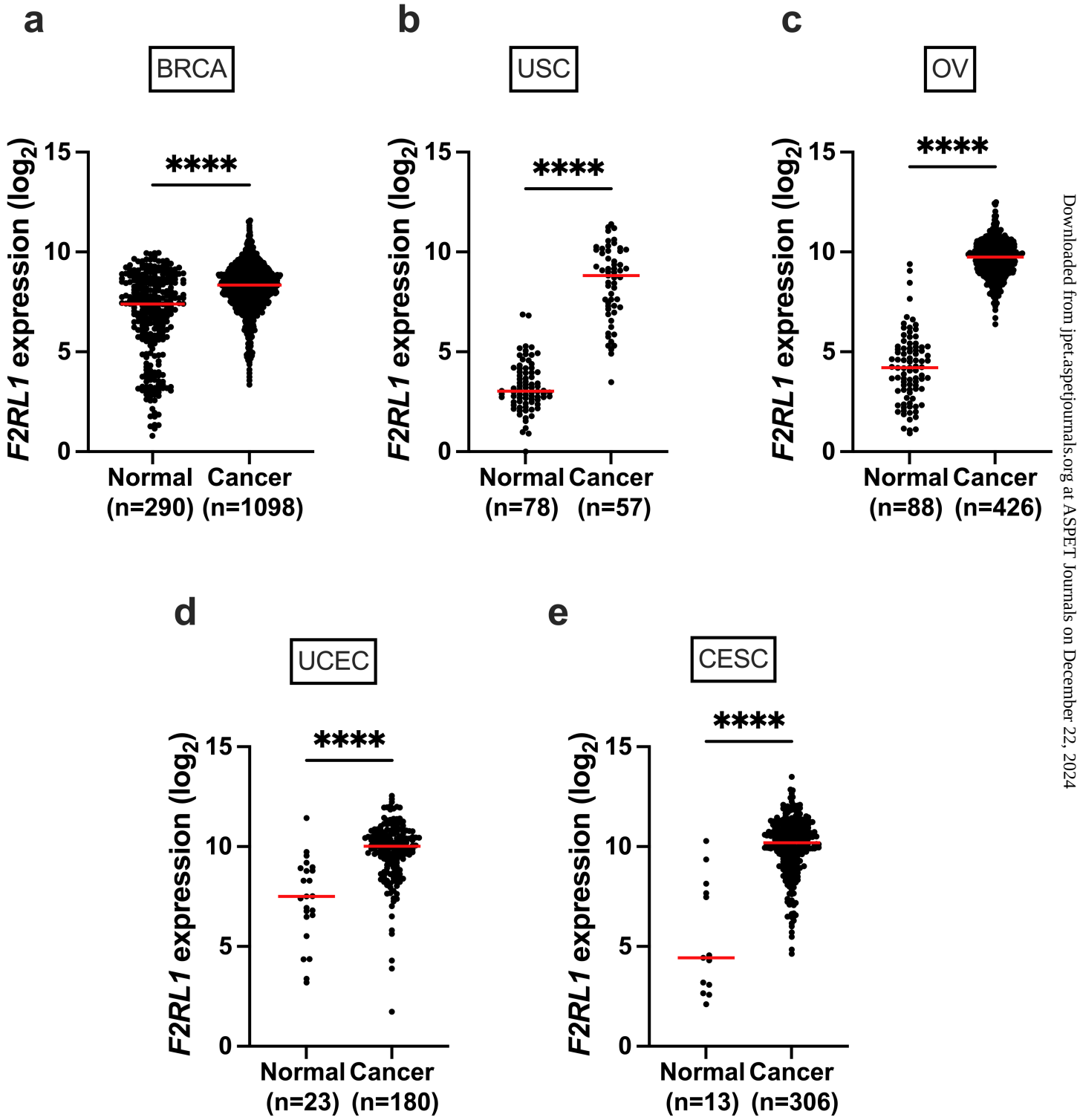


Figure 3

Protease-activated receptor 2 signalling