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Review

Tumour progression and cancer-induced pain: A role for protease-activated receptor-2?

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ABSTRACT

The role of proteases in modifying the microenvironment of tumour cells has long been recognised. With the discovery of the protease-activated receptor family of G protein-coupled receptors a mechanism for cells to sense and respond directly to proteases in their microenvironment was revealed. Many early studies described the roles of protease-activated receptors in the cellular events that occur during blood coagulation and inflammation. More recently, studies have begun to focus on the roles of protease-activated receptors in the establishment, progression and metastasis of a variety of tumours. This review will focus on the expression of protease-activated receptor-2 and its activators by normal and neoplastic tissues, and describe current evidence that activation of protease-activated receptor-2 is an important event at multiple stages of tumour progression and in pain associated with cancer.

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1. Protease-activated receptors

The protease-activated receptor (PAR) family of G protein-coupled seven transmembrane domain receptors were discovered in the early 1990s largely due to their ability to elicit responses from cells stimulated by serine proteases (reviewed in Adams et al., 2011). The four members of this family (PAR_{1-4}) share a common and novel mechanism of activation, whereby proteolytic cleavage of the extracellular amino-terminal domain unmasks a new amino-terminal sequence that binds to a conserved region of the second extracellular loop (Fig. 1). The intramolecular binding of the tethered ligand induces a conformational change in the intracellular, carboxy-terminal domain of the receptor to initiate G protein-dependent signalling via phospholipase C (PLC), intracellular Ca^{2+} mobilisation, protein kinase C (PKC) or mitogen-activated kinase (MAPK)/ERK1/2, or to initiate G protein-independent signalling through recruitment of β-arrestin (Adams et al., 2011). Treatment of cells with synthetic peptides based upon the sequence of

Abbreviations: PAR, Protease-activated receptor; PLC, phospholipase C; PKC, protein kinase C; MAPK, mitogen-activated kinase; TF-FVIIa, tissue factor and factor VIIa; EGFR, epidermal growth factor receptor; VEGF, vascular endothelial growth factor.

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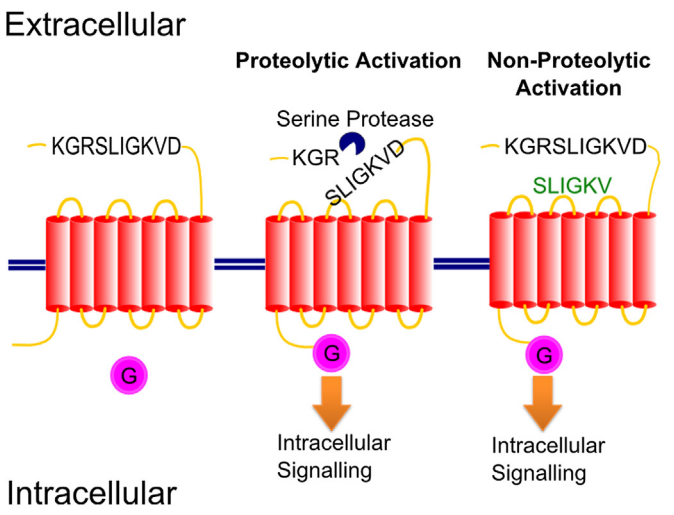

Fig. 1. Structure and activation of protease-activated receptor 2. PAR_2 is a member of the seven-transmembrane domain receptor family of G protein-coupled receptors. Activation of PAR_2 can occur in response to proteolytic cleavage of the peptide bond following a conserved arginine residue in the extracellular domain of the receptor. Proteolysis reveals a new amino terminus that acts as a tethered ligand, binding the second extracellular loop of the molecule and inducing a conformational change in the intracellular carboxy terminal domain that causes recruitment of G proteins and initiation of intracellular signalling cascades. The receptor may also be activated in the absence of proteolysis by short peptides based on the sequence of the tethered ligand (SLIGKV in human PAR_2), which bind the second extracellular loop of the molecule thereby initiating intracellular signalling cascades.

tethered ligands of PAR1, 2 and 4 is sufficient to initiate intracellular signalling from these receptors in the absence of proteolysis (Vergnolle, 2009).

Whereas the other members of the PAR family are largely activated by thrombin, PAR_2 is not. However, PAR_2 is activated by serine and cysteine proteases including trypsin, the blood coagulation proteases tissue factor – factor VIIa complex (TF-VIIa) and factor Xa, mast cell tryptase, neutrophil elastase, kallikreins (KLK)-2, 4, 5, 6 and 14, the transmembrane serine proteases matriptase-1 (TMPRSS14) and TMPRSS2, the mite and cockroach allergens Der P1, 3 and 9 and gingipains from *Porphyromonas gingivalis* (Adams et al., 2011). Cleavage of PAR_2 by trypsin reveals a canonical PAR_2 tethered ligand sequence SLIGKV or SLIGRL (human and mouse PAR_2 single amino acid sequence, respectively) and binding of this sequence to the second extracellular loop of the receptor recruits G proteins that mediate intracellular Ca^{2+} mobilisation (Nystedt et al., 1994). Interestingly, PAR_2 in common with other PARs has been shown to trigger selective cellular effects via the phenomenon of biased agonism. For example, activation of PAR_2 by neutrophil elastase cleaves the extracellular domain downstream of the trypsin recognition site, thereby disarming the G_q-mediated intracellular Ca^{2+} response and initiating a tethered ligand-independent MAPK/ERK1/2 signalling cascade instead (Ramachandran et al., 2011). The ability of PAR_2 to exhibit biased agonism suggests some flexibility in its physiological and pathological functions, which is determined by the proteases in the microenvironment of target cells.

2. Expression of PAR_2 and its agonists in the tumour cell microenvironment

In normal tissues PAR_2 is widely expressed and its expression has been reported on cells including leukocytes, fibroblasts, osteoblasts, myoblasts and muscle fibres, keratinocytes, astrocytes, neurones, glial cells, epithelial cells, endothelial cells and smooth muscle cells (reviewed in Macfarlane et al., 2001; Mackie et al., 2008). PAR_2 expression has also been reported on many cell lines isolated from tumours, including those from lymphoblastic leukaemia, lung, colon, prostate and pancreatic tumours (Bohm et al., 1996), gastric tumours (Miyata et al., 1999), hepatocellular carcinoma (Kaufmann et al., 2009), melanocytic lesions (Massi et al., 2005), breast cancer (D'Andrea et al., 2001) and cervical cancer (Sánchez-Hernández et al., 2008). Furthermore, PAR_2 over expression has been reported in tumours of breast, lung, prostate and stomach (Black et al., 2007; Caruso et al., 2006; Jin et al., 2003; Su et al., 2009). Thus, in many tumours PAR_2 is likely to be expressed by both host tissue and neoplastic cells.

Table 1
Expression of kallikrein agonists of PAR_2 by tumour cells.

Enzyme	Tumour	Reference
KLK4	Breast, ovarian and colorectal cancer	Papachristopoulou et al. (2009)
	Prostate tumours	Klokk et al. (2007)
	Prostate cancer bone metastatic lesions	Ramsay et al. (2008)
KLK5	Breast cancer	Yousef et al. (2003)
	Ovarian cancer	Kim et al. (2001)
KLK6	Pancreatic cancer	Yousef et al. (2004)
	Gastric cancer	Nagahara et al. (2005)
	Colorectal cancers	Kim et al. (2011), Ogawa et al. (2005)

Tumour cells grow within a complex microenvironment, which forms an interface with the host tissue. Into this microenvironment tumour cells secrete an array of proteases, in particular members of the serine protease and matrix metalloproteinase (MMP) families. Expression of many of these proteases has been associated with tumour progression, in part due to their ability to modify the tumour microenvironment by cleaving constituents of the extracellular matrix. Of the serine proteases expressed by tumour cells a number have been shown to be capable of activating PAR_2 and elevated expression of a number of these PAR_2 agonists has been linked to poor prognosis in a variety of tumours (Fujimoto et al., 2006; Kim et al., 2001, 2011; Malfettone et al., 2013; Yamamoto et al., 2003). The best characterised of the PAR_2 agonists is trypsin, which has been reported to be co-localised with PAR_2 expression in the human colon cancer cell lines HT29, T84, Caco-2, and C1.19A (Ducroc et al., 2002), the human cervical carcinoma cell lines SiHa, CasKi, HeLa, UISO-SQC-1 and C-33A (Sánchez-Hernández et al., 2008), as well as in microdissected alveolar walls from human lung adenocarcinoma samples and the human lung adenocarcinoma cell lines LC-2 and PC-14 (Jin et al., 2003).

A second group of PAR_2 agonists reported to be associated with tumours are members of the kallikrein family of secreted serine proteases (Borgono et al., 2004). Of the members of this family that are known to activate human PAR_2 (Mize et al., 2008; Oikonomopoulou et al., 2006; Ramsay et al., 2008), KLK4, KLK5 and KLK6 have been reported to show elevated expression in a variety of primary tumours, including those of pancreas, breast, ovary, colon and prostate as well as in prostate cancer bone metastatic lesions (Table 1).

Other PAR_2 agonists associated with cancers include coagulation proteases, mast cell tryptase and the type II transmembrane serine proteases matriptase-1 (TMPRSS14) and TMPRSS2. The PAR_2-activating complex TF-FVIIa as well as factor Xa have been found to be co-localised in breast cancer, colon cancer and glioma cells (Magnus et al., 2010; Ryden et al., 2010; Zhou et al., 2008), whereas mast cell tryptase expression in the tumour microenvironment has been reported to be associated with infiltrating mast cells in the stroma of colorectal and renal cell carcinoma specimens (Malfettone et al., 2013; Watanabe et al., 2012). Expression of matriptase-1 and TMPRSS2 has been reported in prostate tumours and the human prostate cancer cell lines LNCaP, PC3 and DU-145