ORAL PATHOLOGY

EXPRESSION AND ROLE OF PROTEASE-ACTIVATED RECEPTOR-2 IN CANCER - LITERATURE REVIEW

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ABSTRACT

Protease-activate receptor-2 (PAR-2) is a member of the family of transmembrane receptors with a novel mechanism of activation. Cleavage of the extracellular domain by the ligand enzyme creates a new "tether ligand" that binds back to the receptor to activate it. The ligand enzyme for PAR-2 is trypsin. PAR-2 may also be activated by a short synthetic peptide based on the amino acid sequences of the proteolytically revealed tethered ligand.

PAR-2 is expressed normally in cells like endothelium, keratinocytes, neurons, leukocytes and lung fibroblasts and is present in colon, kidney, liver, pancreas, prostate, skin, small intestine and trachea. It has a documented role in cell differentiation and proliferation. PAR-2 has also been shown to act as a potential growth factor. It is also expressed by a variety of tumour cell lines like adenocarcinoma, prostatic carcinoma and pancreatic carcinoma. The role of PAR-2 and its ligand trypsin has been demonstrated in ovarian, lung, gastric and colonic cancers. However, little is known about its expression in oral squamous cell carcinoma (OSCC). It would be interesting to study the expression pattern and role of PAR-2 in cell differentiation and proliferation related to OSCC.

Key words: PAR-2, trypsin, cell differentiation, proliferation, cancer, oral squamous cell carcinoma.

INTRODUCTION

Protease activated receptors

Cellular homeostasis of oral mucosa and skin is maintained, in part, by a complex network of cell surface receptor. Vu et al (1991)1, 2 identified a new family of cell surface receptors called protease-activated receptors. These receptors have a novel mechanism of activation and a number of structural features common with classical G protein coupled receptors. Cleavage of the extracellular domain by the ligand enzyme creates a new "tether ligand" that binds back to the receptor to activate the receptor. The discovery of the thrombin receptor (PAR-1), led to the cloning of other receptors in this family. The second receptor of this family, PAR-2, is similar to PAR-1, but unlike PAR-1, trypsin and trypsin-like proteases activate it. Also, PAR-2 may be activated by a short synthetic peptide based on the amino acid sequence, of the proteolytically revealed tethered (SLIGKV). More recently two other members of the PAR family have been identified. PAR-3 was discovered in 1998 and both are activated by thrombin3. However, PAR-3 does not generate a signal and appears to act as a co-factor for activation of PAR-4.

PARs have a variety of functional domains and cleavage sites and receptor agonist peptide sequence as shown in Fig-1.

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Cleavage Site

| hPAR-1 | (38-60) LDPR / SFLLRN | Amino acid sequences of PAR's amino-terminal exodomains. | h = human, m = murine. |
| mPAR-2 | PNDKYEPFWEDEE (31-53) SKGR / |
| hPAR-2 | SLIGRL ETQPPITGKGV (33-55) SKGR |
| hPAR-3 | / SLIGKVDGTSHVTGKGV (35-56) LPIK / |
| mPAR-4 | TFRGAP PNSFEFPFSAL (55-78) PNPR / |
| hPApeptides | GYPGKF CANDSDTLEPAS / GYPGQV |

Fig 1. Cleavage sites and PAR agonist peptides.

Protease activated receptor-1

For many years, thrombin was known to be a potent activator of platelets and leukocytes but little was known about the nature of cellular receptor. Vu et al. cloned a functional human thrombin receptor by micro-injection of Xenopus oocytes with mRNA from megakaryocyte-like cell line, which are normally responsive to thrombin. A functional thrombin receptor was detected in oocytes by measuring an intracellular calcium flux in response to thrombin.

Structure of PAR-1

PAR-1, which comprises 425 amino acids, consists of seven helical hydrophobic transmembrane domains and other features common to the G protein-coupled receptors.

Protease-activated receptor-2

Although the cloning of PAR-1 was a major advance in the understanding of the physiological actions of thrombin, the possibility of other serine protease-activated receptors was likely. It had been noted that the effects of thrombin on cells could not be entirely reproduced by the addition of an activating peptides.

PAR-2 was cloned in 1994 by Nystedt et al. in 1994. Human PAR-2 shows 83% amino acid homology with murine PAR-2 and 35% homology with human PAR-1. T receptors 10,12 frame encodes a 365 amino acid protein with seven putative transmembrane domains.

Fig 2. Schematic diagram of PAR-2
calcium. An agonist peptide of sequence SLIGKV-NH₂ was able to produce similar effects. Thrombin or the thrombin receptor agonist peptide had no effect on PAR-2. Detailed studies established the binding site for the tethered ligand and agonist peptide to the second extracellular loop and substitution of this domain in PAR-2 with that of PAR-1 reversed specificity for the respective agonist peptides".

Activation by trypsin and PAR-2 agonist peptide was demonstrated in a variety of cells subsequently shown to express PAR-2, such as intestinal epithelial cells and lung adenocarcinoma cell lines, human keratino-cytes, human umbilical vein endothelial cells, human neutrophils and rat kidney epithelial cells. Therefore, PAR-2 appears to be expressed and functional in a wide variety of cell types in vitro.

Following the initial characterisation of PAR-2 as a trypsin sensitive receptor, whether trypsin itself is the physiological endogenous activator in all tissues remains to be established. High levels of expression of PAR-2 in the small intestine and colon and lower levels of expression in the stomach suggest that potentially, direct activation of PAR-2 by trypsin may occur when it is released from its zymogen precursor, trypsinogen, by enteropeptidase within the duodenum. However this mode of activation restricts the role of trypsin as a PAR-2 activator to the GI tract, except during pancreatitis when trypsin can be released into blood stream. Unexpectedly, recent studies have shown that many cell types also express trypsinogen. These studies indicate that autocrine activation of PAR-2 by trypsinogen may be more widespread than previously thought. This is supported by a recent study indicating that extra-pancreatic tumour associated trypsin-2 can activate PAR-2 in a model system.

Thrombin is unable to activate PAR-2, although trypsin may activate PAR-1, suggesting an overlap in receptor specificity for enzymes. The mast cell serine protease, 'tryptase' was shown to activate PAR-2 expressed by HUVEC and transfected COS-1 cells, but was a poor activator of PAR-1.

A number of other tissue specific enzymes that can activate PAR-2 have now been identified; Fox and coworkers showed the sperm enzyme 'acrosin' more rapidly initiating receptor cleavage than either trypsin or trypase. This finding was consistent with the presence of PAR-2 in oocyte and the epithelium of seminiferous tubules and a recent study that indicates acrosin activation of native PAR-2 on oocytes. Other tissue specific proteases that have been shown to activate PAR-2 include a proteolytic fragment of the neuronal protein B-50/GAP-43, tissue factor Xa, a brain derived serine protease (P22) and gingipain-R, a protease released from P. gingivalis and implicated in adult periodontitis.

It is possible that these tissue-specific activators may be acting in tandem with trypsin, tryptase, or other proteases to fully stimulate the receptor.

**Tissue and cellular distribution of PAR-2**

The tissue distribution and expression pattern of PAR-2 was derived from Northern blot analysis which indicated an abundance of PAR-2 mRNA in kidney, stomach, pancreas, liver, colon, and small intestine. Since the discovery of PAR-2, several in vitro studies have been reported describing the functional stimulation of PAR-2.

Localisation of PAR-2 in different tissues may help to construct a hypothesis about its function. For example, PAR-2 agonist peptide (PAR-2 AP) stimulates a dose and time dependent proliferation of human vascular endothelial cells in vitro and in vivo, PAR-2 promotes leukocyte adhesion and rolling.

The cellular localization of PAR-2 was first described for human keratinocytes and it was subsequently shown to be expressed in vascular endothelial cells, epithelial cells of the gastrointestinal tract, gastric smooth muscle and pancreatic acinar cells.

Recent studies have demonstrated that PAR-2 is expressed by a variety of tumour cell lines, such as A549 (lung adenocarcinoma), Caco2 and SW480 (colon adenocarcinoma), DU145 and PC-3 (prostatic carcinoma, PANC-1 (pancreatic duct cell carcinoma). The observation that trypsin and receptor agonist peptide (PAR-2 AP) stimulate [Ca²⁺] mobilization in tumour cells suggests that PAR-2 expressed by these cells is functional, at least in terms of early signaling.

**PAR-2 mediated intracellular signalling**

It is known that both trypsin and PAR-2 activating peptides stimulate IP3 formation and Ca²⁺ in several
MAP kinases are a family of serine/threonine specific protein kinases, which are involved in the transduction of cell signals by mitogens. There is evidence that MAP kinases are also involved in signaling of PAR-2. Interestingly, trypsin is comparable to platelet derived growth factor in its ability to activate MAP-kinases which suggests that PAR-2 may have role as a growth factor.

**Homeostasis of Oral Mucosa**

The oral mucosa forms an anatomical link between the gastric mucosa and skin. In contrast to skin, oral mucosa produces mucus and may or may not be keratinized. It consists of overlying stratified squamous epithelium.

Many factors are important in maintaining cellular homeostasis in oral mucosa and skin. Cytokines appear to be an important group. A key feature of cytokines is that they interact with specific high affinity cell surface receptors. Cytokines may act in an autocrine, paracrine, or endocrine way on the target cells.

Cells of oral mucosa secrete a variety of cytokines, which form a complex network, the regulation of which is not yet fully understood. During inflammation leucocytes, keratinocytes and fibroblast are capable of producing a panel of pro-inflammatory cytokines such as IL-1, DL-6 and IL-8 and thus, of amplifying the inflammation. On the other hand, cell surface adhesion molecules like integrins have an important role of signal transduction and are involved in the regulation of cell growth, differentiation and development.

Given the increasing evidence that protease activated receptors are important in cellular regulation, it is likely that PARs are involved in cellular homeostasis of oral mucosa.

Recent research into the reasons for me loss of epithelial homeostasis in carcinogenesis has focused on a variety of levels of disruption of regulatory molecules, including receptors, adhesion molecules and cytokine signaling pathways. For example, epidermal growth factor (EGF), transforming growth factor-a, (TGFα) and their receptors have been extensively studied in squamous cell carcinoma.

**Squamous Cell Carcinoma**

Oral cancers, ranks as the sixth most common site for malignant tumours behind, in order, stomach, lung, breast, large bowel and uterine cervix. The majority of oral cancers (over 90%) are squamous cell carcinomas.

With the increasing evidence that proteases are involved in inflammation and coagulation such as thrombin stimulated cells, the possibility that proteases are acting in a way analogous to cytokines and growth factor via specific cell surface receptors has been investigated. Initial studies were focused on thrombin and the search for a cell surface receptor.

**Role of PAR-1 in Cancers**

The recognition that thrombin plays an important role in angiogenesis led to investigate about the role of PAR-1 in tumour formation and metastasis. Thrombin can be synthesised by some tumour cells and PAR-1 is highly expressed in tumour cell lines, and in breast carcinomas. Thrombin promotes tumour cell adhesion to endothelial cells, sub-endothelial matrix, fibronectin, and Von Willibrand factor under static conditions, and platelet-dependent adhesion to endothelial cell under flow conditions. This process is mediated by enhanced expression of α-11bβ 3 and other cell surface molecules such as, P selection. Thrombin also promotes the invasion of aggressive breast tumour cells and is implicated in the development of experimental pulmonary metastasis. PAR-1 is expressed in oral squamous cell carcinoma and thrombin induces matrix metalloproteinases (MMP)-2 and (MMP)-9 activities in metastatic cells. Thus, PAR-1 may contribute to the growth and invasive potential of oral squamous cell carcinoma.

**Role of PAR-2 in Cancers**

Although PAR-1 has been extensively studied, PAR-2 activation may also play a role in regulation of some forms of cancer. Proteolytic enzymes such as matrix metalloproteinases (MMPs) and various serine proteases are known to mediate cancer progression and metastasis. The importance of trypsin, a
major serine protease, has been shown recently in several cancers including tumours in the digestive tract. For example, extra-pancreatic production of trypsin was shown in ovarian\(^5\), lung\(^5\), gastric and colonic tumours.\(^5\) Also the trypsinogen gene is expressed at significant levels in epithelial cells and vascular endothelial cells around gastric tumours.\(^21\) Elevated serum trypsin levels have been reported in some digestive cancers.\(^59\) Overexpression of exogenous trypsinogen cDNA in human gastric cancer cells increases their tumorigenicity in nude mice.\(^22\) Trypsin strongly stimulates proliferation of human colon cancer cells in culture.\(^61\) Serine protease inhibitors suppress carcinogenesis in many different \(in\) \(vivo\) assay systems.\(^62\)

The significance and role of PAR-2 expression in OSCC has not been studied so far. Expression of PAR-1 has been demonstrated in an oral squamous cell carcinoma cell line. However, the magnitude of its importance stands unknown. Further studies in this regard may provide an insight into the mechanism of cell proliferation and differentiation associated with malignant changes in head and neck region.

REFERENCES


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