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Review Article

The role of peroxidasin in solid cancer progression

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Peroxidasin is a heme-containing peroxidase enzyme that plays a vital role in the cross-linking of collagen IV molecules in basement membranes. Collagen IV cross-links are essential for providing structure and mechanical stability throughout tissue development, homeostasis, and wound healing. During cancer progression, the basement membrane is degraded, and proteins typically found in the basement membrane, including peroxidasin and collagen IV, can be found spread throughout the tumour microenvironment where they interact with cancer cells and alter cell behaviour. Whilst peroxidasin is reported to be up-regulated in a number of different cancers, the role that it plays in disease progression and metastasis has only recently begun to be studied. This review highlights the current literature exploring the known roles of peroxidasin in normal tissues and cancer progression, regulators of peroxidasin expression, and the reported relationships between peroxidasin expression and patient outcome in cancer.

Introduction

The basement membrane is a thin layer of highly specialised extracellular matrix that underlies epithelial and endothelial cells. It plays many important roles including providing structural support, determining the polarity of anchored cells, regulating cell growth and differentiation, and acting as a filter to regulate the movement of molecules between the bloodstream and the tissue [1,2]. During injury and tissue repair, the basement membrane also facilitates aligning cells for migration and re-epithelialization [1]. Collagen IV is the most abundant component present and plays an important role in providing structure to the basement membrane. To stabilise the collagen IV network, collagen IV trimers are cross-linked via their NC1 domains through sulfilimine bond formation between the sulfur group of Met93 and the amine nitrogen on hydroxy-Lys211 (Figure 1A) [3]. This cross-linking is mediated by the potent oxidant hypobromous acid (HOBr), generated by the peroxidase enzyme, peroxidasin (PXDN), and relies on the presence of both hydrogen peroxide and bromide [4,5]. This PXDN-catalysed sulfilimine bond formation is an evolutionarily conserved structural feature of collagen IV networks that is known to be essential for tissue development and function [6].

PXDN is a heme-containing enzyme first identified in the cardiovascular system [7]. Other members of the mammalian heme peroxidase family include myeloperoxidase (MPO), eosinophil peroxidase (EPX or EPO) and lactoperoxidase (LPO). Peroxidases utilise hydrogen peroxide to form highly reactive intermediates, which then oxidise halide ions (such as bromide, chloride, and thiocyanate) to form hypohalous acids. Both MPO and EPO are key elements of the innate immune response, while LPO is found in exocrine secretions. The hypohalous acids generated by these enzymes are well-characterised for their antimicrobial properties. PXDN, however, has a unique function in the cross-linking of collagen IV molecules. MPO oxidises Cl^- to generate hypochlorous acid (HOCl), LPO oxidises SCN^- to generate hypothiocyanous acid (HOSCN), whereas, both PXDN and EPO utilise Br^- ions to form hypobromous acid (HOBr) [8–11]. Whilst there is some evidence to suggest that PXDN may have some limited capacity to form HOCl in addition to HOBr [9], this activity is thought to be negligible [8,10].
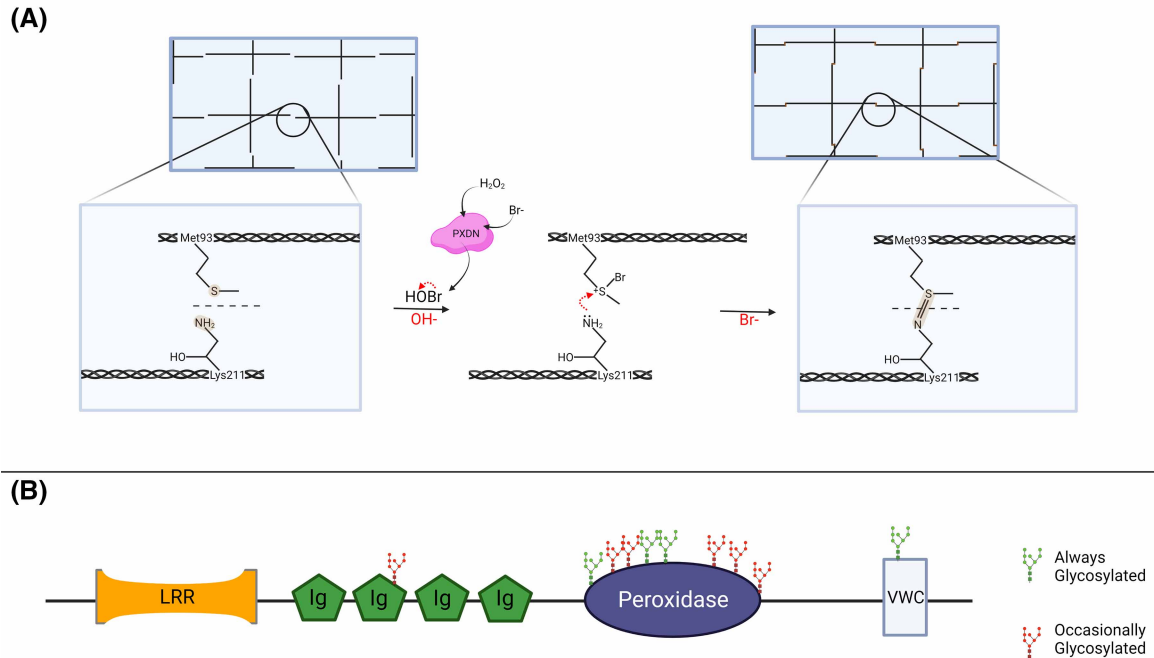
Figure 1. Activity and protein structure of PXDN.

(**A**) HOBr generated by PXDN facilitates formation of sulfilimine cross-links between Met93 and Lys211 on collagen IV molecules. (**B**) Human PXDN contains an LLR domain followed by four repeated Ig domains, a peroxidase domain and the C-terminal VWC domain. There are four sites that have been reported to be always glycosylated, and six additional glycosylation sites that are glycosylated part of the time. Created with BioRender.com.

Importantly, although both PXDN and EPO produce HOBr and both have been shown to promote angiogenesis [12,13], only HOBr generated by PXDN appears efficient in forming the sulfilimine cross-links required to stabilise collagen IV networks [9]. Whilst HOBr from EPO is thought to be able to cross-link some proteins, this is much less efficient than PXDN-mediated generation of sulfilimine bonds within the NC1 domains of collagen IV [14]. Additionally, in *in vitro* studies in which cells were transfected with PXDN, MPO and LPO cDNA and seeded onto uncross-linked basement membrane, only cells transfected with PXDN cDNA were able to generate collagen IV cross-links [9]. More recently, lysyl oxidase like-2 (LOXL-2), an amine oxidase, has been shown to promote the formation of lysyl-derived cross-links in the 7S dodecamer, but not the NC1 domain of collagen IV [6]. Thus, to date, cross-linking of the collagen IV NC1 hexameric junctions appears to be exclusively mediated by PXDN.

In this mini-review, we discuss the known and emerging roles of PXDN in cancer progression, the known associations between PXDN expression and patient outcome, and examine the literature surrounding PXDN regulatory pathways and the implications of these in cancer progression.

PXDN structure and activity

The PXDN enzyme contains four types of domains; the leucine-rich repeat (LLR), immunoglobulin (Ig), peroxidase and von Willebrand factor type C (VWC) domains [8,14–17] (Figure 1B). Both peroxidase and Ig domains are required for optimal sulfilimine bond formation, although studies have shown that bond formation can still occur with the peroxidase domain alone [14]. PXDN is expressed as a monomer, which then assembles into dimers and ultimately a mature homotrimer conformation that is secreted from the cell. These trimers are formed through disulfide bonds, likely between cysteine residues C736 and C1315 [18]. The PXDN protein is also highly glycosylated and has been shown to contain four glycosylation sites and six additional sites that can be glycosylated some of the time (Figure 1B), with glycosylation forming up to 12 kDa of the final mass [8].

The active site required for catalysis of HOBr is in the peroxidase domain which requires heme incorporation for correct function [8]. Association with collagen IV appears to occur through the Ig domains, and is transient [14,15], since a direct binding of PXDN to collagen IV has so far not been shown. However, PXDN has also been shown to be able to interact with laminins through interactions with the leucine-rich repeat domain [15]. This likely facilitates the interaction with collagen IV given their intimately linked roles within the basement membrane, however the exact physiological role for this PXDN-laminin interaction has yet to be determined. Nonetheless, silencing of PXDN expression results not only in a decrease in collagen IV cross-linking, but subsequently reduces laminin and fibronectin content of the basement membrane. Whilst there is no direct evidence to suggest that PXDN cross-links laminin and fibronectin, the close interaction of these with collagen IV suggests that loss of collagen IV network stability has knock-on effects on the biochemistry of the basement membrane.

The emerging role of PXDN in solid cancers

Changes in PXDN expression in cancer

Compared with matched healthy tissue, PXDN is up-regulated in a number of different cancers (Table 1 and Figure 2) including melanoma [19–21], sarcoma [22], glioblastoma [22–24], oral carcinoma [25,26], prostate and testicular cancer [22,27], ovarian cancer [22,28], pancreatic cancer [22,29], thyroid cancer [30], head and neck cancer [22] and stomach cancer [22]. In these studies, PXDN expression has been measured by multiple approaches including mass spectrometry, RT-PCR and/or IHC. Typically PXDN has been shown to increase with advancing stages of disease, such as in prostate [27], testicular [22], colon [22], bladder [22,31], and ovarian cancer [32] (Figure 3). High expression of PXDN has also been reported to be associated with reduced overall survival in ovarian [28], bladder [31], gastric [33,34] and uterine [35] cancers as well as sarcomas [36].

Interestingly, no association has been seen between overall survival and PXDN expression levels in oral squamous cell carcinoma and breast cancer, however in both of these cancers there is reported to be an association between high PXDN expression and poorer disease-free or metastasis-free survival [25,37]. As such, these data suggest a possible role for PXDN in also mediating metastatic dissemination and relapse. There is some evidence to suggest that PXDN expression is highest in mid-stage disease in breast cancer, and begins to decrease again at later stages of disease [38], although what this might mean biologically is unknown. Additional studies stratifying patient outcomes and expression levels of PXDN according to disease stage and/or aggressiveness would help to begin to better understand these differences.

Cell types expressing PXDN in solid cancers

There are limited studies examining the source of expression of PXDN in cancer. PXDN is predominantly expressed by epithelial and endothelial cells in healthy tissue where they are responsible for lens formation in the eye and vascular basement membrane deposition, respectively [16,40,41]. However, PXDN has also been shown to be highly expressed by cancer cells in various cancers [25,27,28,41–45], as well as by some fibroblasts [37] especially during tissue fibrosis [46]. Thus, it is likely that PXDN may be expressed by cancer-associated fibroblasts (CAFs) especially in tumours with a high degree of desmoplasia.

PXDN is highly expressed in cancer cells undergoing epithelial to mesenchymal transition (EMT), a process which underpins metastatic dissemination and poor patient outcomes [47,48]. Whether elevated PXDN expression drives EMT, or is simply a result of EMT, remains to be seen. In a range of melanoma cell lines of varying aggressiveness, PXDN expression was higher in more mesenchymal-like cells [19], which has also been observed in some breast cancer models [37,49]. Therefore, PXDN may be expressed in tumour regions undergoing EMT although the biological mechanisms underpinning this remain to be elucidated. In future, spatial proteomics studies would be highly beneficial in determining the spatial location of PXDN within tumours.

PXDN and collagen IV cross-linking in the tumour microenvironment

The primary known role of PXDN is cross-linking of collagen IV. This is required to provide structural integrity to the basement membrane in normal tissue maintenance and during repair. In cancer, there is commonly a loss of basement membrane integrity. The loss of the basement membrane structure as well as alterations in its biomechanical properties subsequently provides opportunity for increased cell invasion through the basement membrane, a key step associated with metastatic dissemination [50–52]. Collagen IV also becomes aberrantly deposited within the tumour microenvironment, where it interacts with cancer cells and other extracellular matrix molecules to promote disease progression [50–53]. For example, collagen IV is deposited

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Table 1 Expression and function of PXDN in cancer

Cancer type	Expression of PXDN[1]	Reported phenotype	Techniques used	Refs.
Melanoma	Up-regulated	High levels of PXDN increase EMT in melanoma cells.	Comparison of PXDN expression levels in a range of epithelial-like and mesenchymal-like cell lines.	[19,22]
Glioblastoma	Up-regulated	Bioinformatic pan-cancer analysis associating PXDN with patient outcome	Systematic pan-cancer analysis of PXDN using data from TCGA, GTEx, and related databases to compare PXDN expression with clinical characteristics.	[22–24]
Oral carcinomas	Up-regulated	Patients with high PXDN levels had worse disease free survival, lower levels of Reactive Oxygen Species (ROS), increased ATP production and increased lymph node metastasis.	Bioinformatic analysis examining the association between PXDN expression and patient outcomes.	[25,26]
Prostate cancer	Up-regulated	Conditioned media was able to induce migration. Knockdown of PXDN resulted in increased cellular ROS and decreased cell viability and tumorigenicity.	Treatment of cells with conditioned media containing PXDN. shRNA knockdown of PXDN in C4-2 prostate cancer cells	[22,27,65]
Testicular cancer	Up-regulated	Bioinformatic pan-cancer analysis associating PXDN with patient outcome	Systematic pan-cancer analysis of PXDN using data from TCGA, GTEx, and related databases to compare PXDN expression with clinical characteristics.	[22]
Ovarian cancer	Up-regulated	PXDN knockdown reduced proliferation, migration and invasion of cancer cells, as well as reduced PI3K and AKT phosphorylation.	siRNA knockdown of PXDN in HEY ovarian cancer cells.	[22,28]
Pancreatic cancer	Up-regulated	Bioinformatic pan-cancer analysis associating PXDN with patient outcome	Systematic pan-cancer analysis of PXDN using data from TCGA, GTEx, and related databases to compare PXDN expression with clinical characteristics.	[22,29]
Thyroid cancer	Up-regulated	PXDN is part of a four gene signature that showed diagnostic potential in anaplastic thyroid cancer for molecular targeted therapy and immunotherapy.	Exploratory study using ATC RNA-sequencing data from the GEO database, linking PXDN expression to immune infiltration	[30]
Head and neck cancer	Up-regulated	Bioinformatic pan-cancer analysis associating PXDN with patient outcome	Systematic pan-cancer analysis of PXDN using data from TCGA, GTEx, and related databases to compare PXDN expression with clinical characteristics.	[22]
Stomach cancer	Up-regulated	Bioinformatic pan-cancer analysis associating PXDN with patient outcome	Systematic pan-cancer analysis of PXDN using data from TCGA, GTEx, and related databases to compare PXDN expression with clinical characteristics.	[22]
Liver (Hepatocellular Carcinoma)	Up-regulated	Bioinformatic pan-cancer analysis associating PXDN with patient outcome	Systematic pan-cancer analysis of PXDN using data from TCGA, GTEx, and related databases to compare PXDN expression with clinical characteristics.	[22]

Table 1 Expression and function of PXDN in cancer

Cancer type	Expression of PXDN[1]	Reported phenotype	Techniques used	Refs.
Uterine serous carcinoma	Up-regulated	Bioinformatic pan-cancer analysis associating PXDN with patient outcome	Systematic pan-cancer analysis of PXDN using data from TCGA, GTEx, and related databases to compare PXDN expression with clinical characteristics.	[22]
Bladder cancer	Down-regulated	Bioinformatic pan-cancer analysis associating PXDN with patient outcome	Systematic pan-cancer analysis of PXDN using data from TCGA, GTEx, and related databases to compare PXDN expression with clinical characteristics.	[22]
Uterine corpus endometrial carcinoma	Down-regulated	Bioinformatic pan-cancer analysis associating PXDN with patient outcome	Systematic pan-cancer analysis of PXDN using data from TCGA, GTEx, and related databases to compare PXDN expression with clinical characteristics.	[22]
Breast cancer	No significant change	Conditioned media was able to induce migration. Knockdown of PXDN resulted in decreased cell proliferation. Pateints with high PXDN levels had poorer metastasis free survival	Treatment of cells with conditioned media containing PXDN. siRNA knockdown of PXDN in MCF10A cells. Bioinformatic analysis examining the association bewteen PXDN expression and pateint outcomes.	[22,37,65,76]
Colon cancer	No significant change	Bioinformatic pan-cancer analysis associating PXDN with patient outcome	Systematic pan-cancer analysis of PXDN using data from TCGA, GTEx, and related databases to compare PXDN expression with clinical characteristics.	[22]
Lung carcinoma	N/A	Fine particulate matter in air pollution activates PXDN to increase collagen IV cross-linking and decreased cytotoxic T lymphocyte migration in lung cancer	Intra-tracheal delivery of shRNA targeting PXDN into the lungs of mice.	[66]

[1]Expression in comparison with healthy tissue of the same tissue type.

around pancreatic cancer cells encapsulating them within a pseudo-basement membrane-like structure [54,55] (Figure 3). The arrangement of collagen IV may also directly contribute to tumour progression and patient outcome, since thick filament bundle of collagen IV around clusters of cells is observed in well-differentiated cancers, which typically have poor prognosis, whilst non-differentiated models exhibit an arrangement of collagen IV with thinner fibres around individual tumour cells [55].

Potential alternative roles for PXDN and their implications in cancer

PXDN effects on cells are most likely through its collagen IV cross-linking activity, yet there is evidence to suggest that PXDN may have additional functions relevant to the cancer setting. For example, the capacity of the LRR domain in PXDN to interact with laminin [15] has raised questions about a possible role for PXDN in the oxidative modification of laminin and other nearby ECM components, especially given that HOBr is known to cross-link proteins. PXDN may also play a role in heme oxygenase-1 (HO-1) mediated adhesion of BeWo LMP cells to laminin and fibronectin matrices [45], suggesting that PXDN may have the capacity to influence adhesion to other ECM components, however, it is not clear what the mechanism responsible for this is.

Alternately, some studies have hypothesised that PXDN's use of hydrogen peroxide to catalyse the formation of HOBr, both of which are forms of reactive oxygen species (ROS), may be altering the levels of oxidative stress experienced by cancer cells. In non-cancerous aortic and endothelial cells, knockdown of PXDN with siRNA or shRNA led to a decrease in superoxide and ROS levels during oxidative stress [41,56]. In prostate cancer, however, the opposite trend was observed, with PXDN depletion in C4-2 cancer cells resulting in an
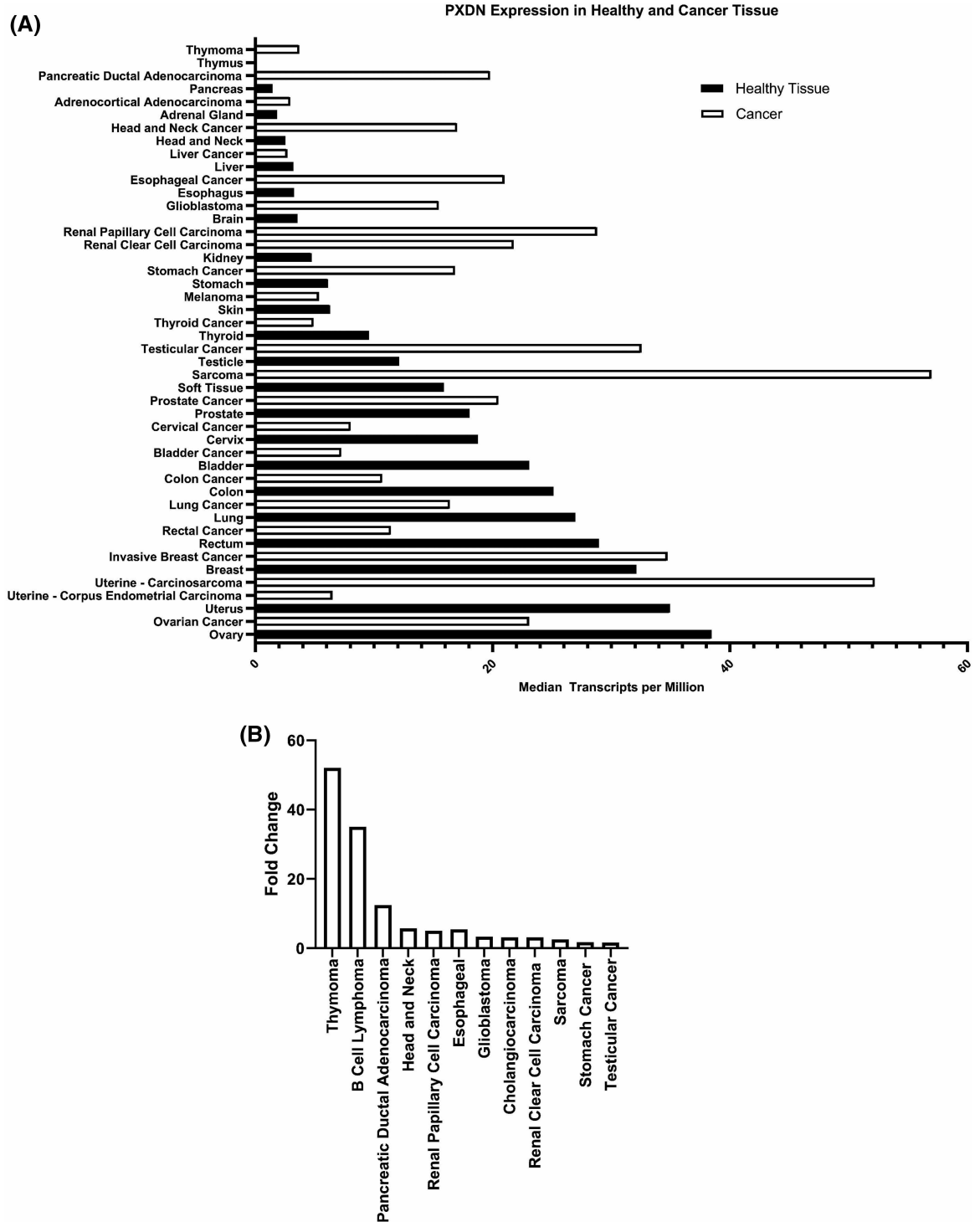
(A)

PXDN Expression in Healthy and Cancer Tissue

Legend:
- ■ Healthy Tissue
- □ Cancer

X-axis: Median Transcripts per Million

Categories (top to bottom): Thymoma, Thymus, Pancreatic Ductal Adenocarcinoma, Pancreas, Adrenocortical Adenocarcinoma, Adrenal Gland, Head and Neck Cancer, Head and Neck, Liver Cancer, Liver, Esophageal Cancer, Esophagus, Glioblastoma, Brain, Renal Papillary Cell Carcinoma, Renal Clear Cell Carcinoma, Kidney, Stomach Cancer, Stomach, Melanoma, Skin, Thyroid Cancer, Thyroid, Testicular Cancer, Testicle, Sarcoma, Soft Tissue, Prostate Cancer, Prostate, Cervical Cancer, Cervix, Bladder Cancer, Bladder, Colon Cancer, Colon, Lung Cancer, Lung, Rectal Cancer, Rectum, Invasive Breast Cancer, Breast, Uterine - Carcinosarcoma, Uterine - Corpus Endometrial Carcinoma, Uterus, Ovarian Cancer, Ovary

(B)

Y-axis: Fold Change (0–60)

Categories: Thymoma, B Cell Lymphoma, Pancreatic Ductal Adenocarcinoma, Head and Neck, Renal Papillary Cell Carcinoma, Esophageal, Glioblastoma, Cholangiocarcinoma, Renal Clear Cell Carcinoma, Sarcoma, Stomach Cancer, Testicular Cancer

Figure 2. PXDN expression levels in healthy and tumour tissue.

Graphs show RNA sequencing data of healthy tissue and corresponding tumour tissue according to data from TCGA and GTEx (collected from GEPIA [39]). (**A**) Median PXDN transcripts per million measured by RNA sequencing. (**B**) Top 12 cancers with the largest fold change in PXDN expression levels in tumour tissue compared with healthy tissue.
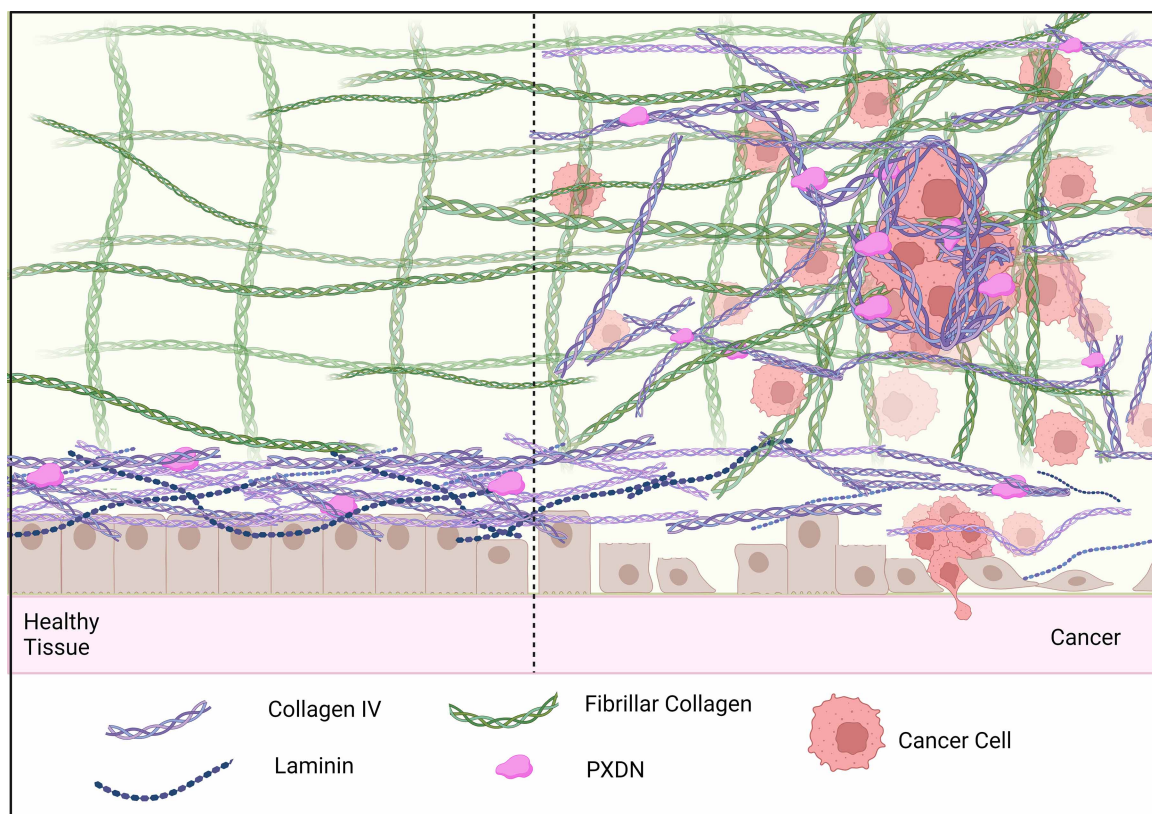
Figure 3. Changes in basement membrane and ECM during cancer progression.
In solid tumours, there is a loss of basement membrane integrity and structure facilitating increased cell invasion into the vasculature and lymphatic systems. Simultaneously, the ECM of the tumour microenvironment becomes dysregulated with increased and aberrant deposition of fibrillar collagens as well as network forming collagens such as collagen IV. PXDN levels are also often elevated within solid tumours, and in light of the known collagen IV cross-linking function of PXDN, this may lead to stablisation of aberrant collagen IV networks within the tumour microenvironment. Created with BioRender.com.

increase in cellular ROS [27]. Furthermore, in oral squamous cell carcinoma, ROS production was found to have an inverse relationship with PXDN expression [25].

PXDN driven changes in oxidative stress may also be mediated through alterations in cellular energetics and metabolism. Purine metabolism is known to be increased in cells to mitigate oxidative stress [57]. Uric acid, an end product of purine metabolism, has been reported to be a possible inhibitor of PXDN [58,59]. Moreover, high levels of PXDN have also been associated with increased ATP production [25], a typical hallmark of cancer metabolism, however there is to our knowledge no direct link between ATP production and PXDN activity so it is likely that there may be confounding variables which were not identified in this study.

Treatment of cells with excess exogenous fatty acids, such as palmitate, is known to trigger apoptotic cell death through impairment of autophagic flux. In cardiomyocytes treated with palmitate, increased levels of PXDN were observed, as well as reduced glucose consumption and impairment of autophagic flux, resulting in higher oxidative stress and subsequent cell death [60]. siRNA depletion of PXDN in these cells improved autophagic flux and consequently reduced cell death. Given that cellular energetics are often altered in cancer cells, future studies could examine the link between glucose metabolism, autophagic flux, oxidative stress and PXDN in the cancer setting.

Mechanisms of regulation of PXDN expression and activity
Regulation of PXDN during normal tissue homeostasis
Due to the critical role of the basement membrane in tissues and the importance of correctly cross-linked collagen IV, the regulation of PXDN is key to basement membrane integrity. The exact mechanisms and pathways that regulate PXDN expression are yet to be fully understood. At the post-transcriptional level, the

2′-O-methylase *Fbl* has been shown to modify PXDN mRNA when guided by small nucleolar RNAs U32A and U51 (Figure 4), which both have high complementarity to a conserved coding region of PXDN mRNA [61]. mRNA methylation has also been shown to decrease translation and production of PXDN [61]. In other work, the microRNA miR-29b-3p, which is known to be up-regulated during cell stress, has been shown to increase PXDN expression levels in cardiac cells *in vitro* [62].

The immature PXDN protein is post-translationally modified to form mature PXDN before secretion from the cell (Figure 4). The C-terminus of PXDN is cleaved at arginine 1336 by the pro-protein convertase furin, resulting in the removal of a 30 kDa propeptide [63,64]. Full length PXDN has been shown to be able to generate HOBr and cross-link collagen IV when added exogenously to cultures *in vitro*, however this activity is significantly higher in the mature, processed form of PXDN [63,64]. Whilst full length PXDN can be detected both intracellularly and extracellularly, only cleaved PXDN is located extracellularly suggesting that proteolytic cleavage occurs at a late stage, just prior to secretion [63]. In support of this, addition of an ER retention signal (KDEL) to the C-terminus of PXDN significantly reduced its secretion by PFHR-9 cells, in turn reducing collagen IV cross-linking [63].

Regulators of PXDN enzymatic activity are not well understood, however physiological levels of thiocyanate and urate have been shown to partially inhibit PXDN cross-linking activity [5]. Given that urate is an end product in purine metabolism (discussed above), and this metabolic pathway is increased in cells undergoing oxidative stress, it is possible that urate may regulate PXDN during oxidative stress [57].

Effects of PXDN dysregulation in tumour progression

The exact role that PXDN plays in the progression of different cancers remains unclear and is likely multifaceted. Abnormal expression of PXDN in tumours has been linked to changes in cell viability, proliferation, migration, and invasion. Elevated PXDN was among the main ECM effectors identified in conditioned media from bone marrow derived mesenchymal stromal cells that induced migration of both PC3 prostate and MDA-MB-231 breast cancer cells [65]. Short hairpin RNA (shRNA) depletion of PXDN in C4-2 prostate cancer cells also reduced cell viability and colony forming ability in soft agar [27]. In ovarian cancer patient tumours, high expression of PXDN is associated with smaller tumours [32], yet *in vitro* depletion of PXDN in HEY human ovarian cancer cells reduced proliferation and migration [28]. Increased levels of PXDN in oral squamous cell carcinoma have also been associated with increased lymph-node metastasis and infiltration at the primary site [25], and high expression of PXDN is associated with shorter metastasis-free survival in breast cancer [37]. These data highlight how the tumour type, source of PXDN (autocrine vs. paracrine), setting (*in vitro* vs. *in vivo* vs. patient) as well as experimental setup may yield contrasting results.

PXDN mediated cross-linking of collagen IV has been linked to changes in immune infiltration, which may play an important role in solid tumour progression. Intra-tracheal delivery of PXDN shRNA into the lungs of mice reduced collagen IV cross-linking and subsequently increased cytotoxic T lymphocyte migration into the lungs [66]. These data strongly suggest that PXDN and/or collagen IV cross-linking may be important in altering the tumour microenvironment and therefore immune cell infiltration in some solid cancers, although this has yet to be tested formally.

Expression of PXDN has been linked to vascular tube formation and angiogenesis in endothelial cells [12,13]. Angiogenesis is crucial in solid tumours as it enables the formation of new blood vessels, providing tumours with nutrients and oxygen, fuelling their growth and metastasis. However, a role for PXDN in tumour angiogenesis has yet to be investigated.

Transcriptional (Dys)-regulation of PXDN expression in cancer

A range of transcriptional regulatory pathways of PXDN expression have been identified in different cancer cells, including methylation, transcription factors and microRNAs (Figure 4). Methylation of the promoter region of PXDN appears to suppress transcription and is negatively correlated with PXDN expression in cancers of the liver, breast, skin, kidney, oesophagus, thyroid, rectum, lung, bladder, stomach, bone, head and neck, colon, prostate, mesothelium, pancreas, uterus, cervix and ovary [22]. In prostate cancer, PXDN promoter methylation has been shown to be mediated through PRMT5 leading to an increase in expression of PXDN [67]. Contrary to these results, methylation frequency of PXDN appears to increase with disease progression in pancreatic cancer [68], whilst methylation status of PXDN showed no significant association with patient outcomes in colorectal cancer [69].

PXDN typically exhibits a lower mutational burden than many common oncogenes. However, mutations in the PXDN gene appear frequently in uterine cancers [22], stomach cancer [22], melanoma [21,22] and colon
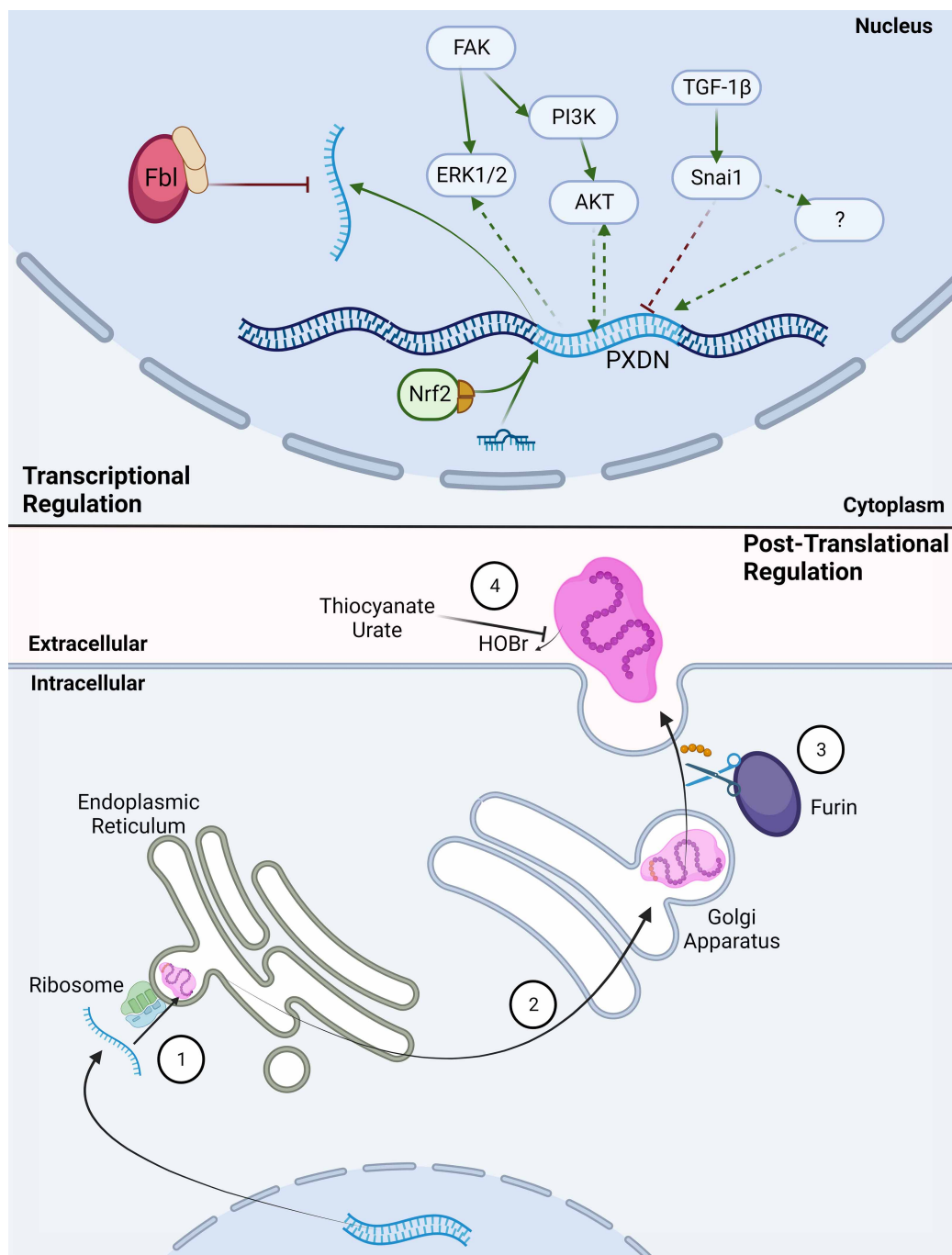
Figure 4. Regulation of PXDN at the transcriptional and post-translational level.

At the transcriptional level, PXDN expression can be increased by binding of nuclear factor Nrf2 when joined with inducers tBHQ and SFN, or microRNAs miR-203 or miR-29b-3p, to the promoter region of the PXDN gene. The Snai1, FAK/PI3K/AKT and FAK/ERK signalling pathways have all been linked to regulation of PXDN expression, although the exact mechanisms underlying this regulation have yet to be properly understood (dotted lines). The 2′-O-methylase Fbl, guided by snorU32A and U52, can methylate PXDN mRNA to reduce levels of transcription. Once PXDN mRNA exits the nucleus, it is co-translationally inserted into the ER as full length, immature protein (1), before being transported through the endoplasmic reticulum and golgi apparatus via the classical secretory pathway (2) and secreted from the cell. Prior to secretion, the c-terminal peptide is cleaved from the full length PXDN protein by the pro-protein convertase Furin to form the mature PXDN protein (3). Activity of the mature PXDN protein can be moderated by thiocyanate and urate, which act as alternative substrates for PXDN, resulting in reduced HOBr production and therefore reduced cross-linking activity (4). Created with BioRender.com.

cancer [22,70]. The most common type of mutations of the PXDN gene in cancers are missense mutations [21,22,36,70], however the pathology of many of these, including potential effects on enzymatic activity, if any, remains unknown. Survival analyses performed in cohorts of melanoma patients found no direct association between mutations in the PXDN gene and overall survival [21], yet Paumann-Page et al. [20] recently demonstrated a correlation of PXDN expression and survival in melanoma. However, with limited or no functional validation to date, it is not clear whether various mutations in the PXDN gene would alter expression or activity.

The nuclear factor Nrf2 has been shown to increase PXDN expression by HeLa cells through binding to the PXDN promoter region [71]. The Nrf2-mediated oxidative response pathway was also among the most differentially regulated between PXDN knockdown and controls in C4-2 prostate cancer cells [27]. Additionally, a binding site for the microRNA miR-203a has been identified in the 3′ UTR of PXDN [72] suggesting that PXDN may fall under miR regulation, and supporting work has shown that addition or removal of miR-203a was able to increase or decrease expression of PXDN respectively. Complementing this, PXDN has also been predicted to be a target gene of miR-203 in pancreatic cancer [73].

PXDN regulation in cancer may also be a result of dysregulation of Snai1, a transcriptional repressor. ChiP-PCR analysis and functional assays in HeLa and SiHa cells showed binding of Snai1 to the PXDN promoter region upon TGF-b1 stimulation [43] led to repression of PXDN expression. In contrast, in a panel of prostate cancer cell lines, PXDN protein levels were found to be higher in cells expressing Snai1 and vimentin [27]. Additionally, in ARCaP prostate cancer cells transfected with Snai1 cDNA to overexpress Snai1, PXDN expression was significantly fold up-regulated compared with control [42].

Post-translational (Dys)-regulation of PXDN expression in cancer

The exact regulatory mechanisms of PXDN during homeostasis are not yet fully understood. For this reason, further research into this area in cancer, particularly in comparison with healthy tissue, is needed. A better understanding of the cancer-specific alterations in PXDN expression and activity, the downstream effects of collagen IV cross-linking, and role this plays in disease progression is needed.

Modulation of intracellular signalling in response to PXDN dysregulation

Kinase signalling plays a pivotal role in cancer progression. Dysregulation of extracellular cues, such as aberrant matrix cross-linking, impact significantly on downstream intracellular signalling networks [74]. Whilst investigation into the role of PXDN on intracellular signalling in cancer is limited, research in non-transformed cells has implicated PXDN in the regulation of key signalling networks. For example, endothelia cells transfected with PXDN siRNA show reduced migration, proliferation and survival *in vitro* [75] mediated through decreased phosphorylation in ERK/Akt/FAK signalling. Exogenous addition of active forms of purified recombinant PXDN (rPXDN) to TeloHAEC cardiac epithelial cells increased phosphorylation of Akt, ERK1/2 and FAK, whilst addition of a catalytically inactive form of PXDN had no effect [13].

PXDN may also play a role in modulating PI3K/Akt signalling in cancer (Figure 4). In U87.MGΔEGFR glioblastoma cells with constitutive expression of Epidermal growth factor receptor (EGFR) — a known regulator of PI3K/Akt signalling — PXDN was the most highly up-regulated gene [44]. siRNA knockdown of PXDN in HEY ovarian cancer cells also reduced phosphorylation of PI3K/Akt, leading to reduced invasion and migration [28]. However, whether this reduction in cell invasiveness was the result of changes in PI3K/Akt signalling was not examined. On the other hand, breast cancer cells with siRNA knockdown of PXDN showed decreased proliferation whilst maintaining Akt and ERK phosphorylation [76].

Other signalling pathways that have been shown to be modulated in endothelial cells by rPXDN protein include PDGFb, HB-EGF, CXCL, Hey-1, ID-2, SNAI-1 [13], FAK and ERK1/2 [75]. PXDN depletion has also been shown to reduce ERK signalling in hypoxia treated pulmonary artery smooth muscle cells [77] and attenuated a hydrogen peroxide induced increase in ERK1/2 phosphorylation in vascular smooth muscle cells [78]. Further studies are required however to determine the effects of PXDN expression in the solid tumour setting where many of these signalling pathways are also dysregulated.

Considerations for therapeutic targeting of PXDN
Effects of PXDN expression levels on current cancer treatments

There is evidence to suggest that PXDN may influence tumour sensitivity to therapies. The IC$_{50}$'s of several kinase inhibitors were all reported to be higher in cell lines with high PXDN expression compared with low

PXDN. However these results were not separated for different cancer types [22]. Additionally, in the PyMT mouse model of breast cancer, treatment with paclitaxel or doxorubicin resulted in remodelling of the ECM which included an observed increase in collagen IV abundance [79]. Whilst cross-linking was not measured, this increase in collagen IV presence appeared to induce invasion of PyMT cells, and invasion could be attenuated upon the administration of clinical FAK inhibitors [79]. Given that PXDN depletion using siRNA reduced FAK phosphorylation in HUVEC cells, it could be speculated that PXDN expression and/or activity, and FAK signalling may work together to play a role in the invasion of breast cancer cells, although the direct interplay between FAK inhibitors and PXDN expression and activity should be further explored in this setting.

Possible future therapeutics targeting PXDN in cancer

The increased expression of PXDN in many cancers, as well as its links to increased cell proliferation, migration, invasion, and metastasis, suggest that it may be a viable candidate for therapeutic targeting in cancer. At present, no PXDN specific inhibitors have been developed. There are, however, a number of inhibitors that target peroxidases more broadly which have been shown to inhibit PXDN activity, including 4-aminobenzoic acid hydrazide (4-ABAH) [5,7,80], phloroglucinol (PHG) [4,5,9,59,75,81,82], methimazole [9,83] and 3-aminotriazole [9,83]. Additionally, collagen IV cross-linking is partially inhibited by urate [58,59], iodide [5,9] and thiocyanate [5].

A key consideration will be the broad inhibition of multiple members of the peroxidase family including MPO and EPO, both of which are known to play important roles in solid tumours, contributing to tumour growth, collagen deposition and metastasis [84]. Deficiencies in MPO and EPO are not known to lead to negative health effects [85,86], however both MPO and EPO have been shown to be internalised by fibroblasts, increasing their collagen deposition and migration [87], and endothelial cells resulting in increased proliferation, invasion and angiogenesis [12]. In this context, inhibitors targeting MPO, EPO and PXDN may have favourable outcomes for treating cancer compared with targeting single peroxidase enzymes alone.

Given that PXDN mediated cross-linking of collagen IV is an essential process in basement membrane generation and repair, inhibition of this process outside of the tumour microenvironment may have negative effects. PXDN does not appear to be essential in development since mice with homozygous deletion of PXDN present with eye defects but no other severe morphological changes [88]. However, the depletion of PXDN in the context of wound healing should be carefully considered. Targeting of peroxidase inhibitors specifically to the tumour microenvironment may be able to reduce the impact of these potential undesirable effects.

An alternative approach would be to target post-translational regulators of PXDN. This approach has been trialled *in vitro* with inhibitors such as CMK [63], decanoyl-RVKR-chloromethyl ketone and a-1 antitrypsin Portland variant [64], which target Furin and other pro-protein convertases, as well as by utilising methionine as a hypohalous acid scavenger [80]. Each of these approaches will likely exhibit pleiotropic effects on tissue homeostasis and disease, and so the potential impact of off-target effects should be carefully considered.

Ultimately, more research is needed to understanding the exact role that PXDN plays in different cancer types and how this is regulated before researchers and pharmaceutical companies consider PXDN to be an economically viable target for the development of targeted therapies.

Perspectives

- Peroxidasin is an enzyme with an emerging role in tumour progression and metastasis that is involved in extracellular matrix (ECM) remodelling and modulating the composition and organisation of the ECM.

- Peroxidasin promotes cancer cell invasion, angiogenesis, and has been associated with increased metastasis.

- Further research is required to better understand the molecular mechanisms of peroxidasin in cancer progression, as well as downstream signalling pathways and interacting partners of peroxidasin. This will allow for the development of targeted therapies for cancer treatment.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EMT, epithelial to mesenchymal transition; HOBr, hypobromous acid; HOCl, hypochlorous acid; LLR, leucine rich repeat; LPO, lactoperoxidase; MPO, myeloperoxidase; PXDN, peroxidasin; ROS, reactive oxygen species; VWC, von Willebrand factor type C.

References

1 Mak, K.M. and Mei, R. (2017) Basement membrane type IV collagen and laminin: an overview of their biology and value as fibrosis biomarkers of liver disease. *Anat. Rec. (Hoboken)* **300**, 1371–1390 https://doi.org/10.1002/ar.23567

2 Khalilgharibi, N. and Mao, Y. (2021) To form and function: on the role of basement membrane mechanics in tissue development, homeostasis and disease. *Open Biol.* **11**, 200360 https://doi.org/10.1098/rsob.200360

3 Vanacore, R., Ham, A.-J.L., Voehler, M., Sanders, C.R., Conrads, T.P., Veenstra, T.D. et al. (2009) A sulfilimine bond identified in collagen IV. *Science* **325**, 1230–1234 https://doi.org/10.1126/science.1176811

4 Bathish, B., Paumann-Page, M., Paton, L.N., Kettle, A.J. and Winterbourn, C.C. (2020) Peroxidasin mediates bromination of tyrosine residues in the extracellular matrix. *J. Biol. Chem.* **295**, 12697–12705 https://doi.org/10.1074/jbc.RA120.014504

5 Bathish, B., Turner, R., Paumann-Page, M., Kettle, A.J. and Winterbourn, C.C. (2018) Characterisation of peroxidasin activity in isolated extracellular matrix and direct detection of hypobromous acid formation. *Arch. Biochem. Biophys.* **646**, 120–127 https://doi.org/10.1016/j.abb.2018.03.038

6 Añazco, C., López-Jiménez, A.J., Rafi, M., Vega-Montoto, L., Zhang, M.-Z., Hudson, B.G. et al. (2016) Lysyl oxidase-like-2 cross-links collagen IV of glomerular basement membrane. *J. Biol. Chem.* **291**, 25999–26012 https://doi.org/10.1074/jbc.M116.738856

7 Cheng, G., Salerno, J.C., Cao, Z., Pagano, P.J. and Lambeth, J.D. (2008) Identification and characterization of VPO1, a new animal heme-containing peroxidase. *Free Radic. Biol. Med.* **45**, 1682–1694 https://doi.org/10.1016/j.freeradbiomed.2008.09.009

8 Soudi, M., Paumann-Page, M., Delporte, C., Pirker, K.F., Bellei, M., Edenhofer, E. et al. (2015) Multidomain human peroxidasin 1 is a highly glycosylated and stable homotrimeric high spin ferric peroxidase. *J. Biol. Chem.* **290**, 10876–10890 https://doi.org/10.1074/jbc.M114.632273

9 Bhave, G., Cummings, C.F., Vanacore, R.M., Kumagai-Cresse, C., Ero-Tolliver, I.A., Rafi, M. et al. (2012) Peroxidasin forms sulfilimine chemical bonds using hypohalous acids in tissue genesis. *Nat. Chem. Biol.* **8**, 784–790 https://doi.org/10.1038/nchembio.1038

10 Paumann-Page, M., Katz, R.-S., Bellei, M., Schwartz, I., Edenhofer, E., Sevcnikar, B. et al. (2017) Pre-steady-state kinetics reveal the substrate specificity and mechanism of halide oxidation of truncated human peroxidasin 1. *J. Biol. Chem.* **292**, 4583–4592 https://doi.org/10.1074/jbc.M117.775213

11 Colon, S., Page-McCaw, P. and Bhave, G. (2017) Role of hypohalous acids in basement membrane homeostasis. *Antioxid Redox Signal.* **27**, 839–854 https://doi.org/10.1089/ars.2017.7245

12 Panagopoulos, V., Zinonos, I., Leach, D.A., Hay, S.J., Liapis, V., Zysk, A. et al. (2015) Uncovering a new role for peroxidase enzymes as drivers of angiogenesis. *Int. J. Biochem. Cell Biol.* **68**, 128–138 https://doi.org/10.1016/j.biocel.2015.09.006

13 Medfai, H., Khalil, A., Rousseau, A., Nuyens, V., Paumann-Page, M., Sevcnikar, B. et al. (2019) Human peroxidasin 1 promotes angiogenesis through ERK1/2, Akt, and FAK pathways. *Cardiovasc. Res.* **115**, 463–475 https://doi.org/10.1093/cvr/cvy179

14 Ero-Tolliver, I.A., Hudson, B.G. and Bhave, G. (2015) The ancient immunoglobulin domains of peroxidasin are required to form sulfilimine cross-links in collagen IV. *J. Biol. Chem.* **290**, 21741–21748 https://doi.org/10.1074/jbc.M115.673996

15 Sevcnikar, B., Schaffner, I., Chuang, C.Y., Gamon, L., Paumann-Page, M., Hofbauer, S. et al. (2020) The leucine-rich repeat domain of human peroxidasin 1 promotes binding to laminin in basement membranes. *Arch. Biochem. Biophys.* **689**, 108443 https://doi.org/10.1016/j.abb.2020.108443

16 Cheng, G. and Shi, R. (2022) Mammalian peroxidasin (PXDN): from physiology to pathology. *Free Radic. Biol. Med.* **182**, 100–107 https://doi.org/10.1016/j.freeradbiomed.2022.02.026

17 Paumann-Page, M., Tscheliessnig, R., Sevcnikar, B., Katz, R.-S., Schwartz, I., Hofbauer, S. et al. (2020) Monomeric and homotrimeric solution structures of truncated human peroxidasin 1 variants. *Biochim. Biophys. Acta Proteins Proteom.* **1868**, 140249 https://doi.org/10.1016/j.bbapap.2019.07.002

18 Lázár, E., Péterfi, Z., Sirokmány, G., Kovács, H.A., Klement, E., Medzihradszky, K.F. et al. (2015) Structure-function analysis of peroxidasin provides insight into the mechanism of collagen IV crosslinking. *Free Radic. Biol. Med.* **83**, 273–282 https://doi.org/10.1016/j.freeradbiomed.2015.02.015

19 Jayachandran, A., Prithviraj, P., Lo, P.-H., Walkiewicz, M., Anaka, M., Woods, B.L. et al. (2016) Identifying and targeting determinants of melanoma cellular invasion. *Oncotarget* **7**, 41186–41202 https://doi.org/10.18632/oncotarget.9227

20 Paumann-Page, M., Kienzl, N.F., Motwani, J., Bathish, B., Paton, L.N., Magon, N.J. et al. (2021) Peroxidasin protein expression and enzymatic activity in metastatic melanoma cell lines are associated with invasive potential. *Redox Biol.* **46**, 102090 https://doi.org/10.1016/j.redox.2021.102090

21 Carvalho, L.A.C., Queijo, R.G., Baccaro, A.L.B., Siena, Á.DD., Silva, W.A., Rodrigues, T. et al. (2022) Redox-Related proteins in melanoma progression. *Antioxidants (Basel)* **11**, 438 https://doi.org/10.3390/antiox11030438

22 Zhou, X., Sun, Q., Xu, C., Zhou, Z., Chen, X., Zhu, X. et al. (2022) A systematic pan-cancer analysis of PXDN as a potential target for clinical diagnosis and treatment. *Front. Oncol.* **12**, 952849 https://doi.org/10.3389/fonc.2022.952849

23 Kumari, A.S. (2022) Identification and analysis of molecular pathways and key candidate biomarkers in glioblastoma multiforme. *Res Sq.* https://doi.org/10.21203/rs.3.rs-2127048/v1

24 Ghochani, Y., Muthukrishnan, S.D., Sohrabi, A., Kawaguchi, R., Condro, M.C., Bastola, S. et al. (2022) A molecular interactome of the glioblastoma perivascular niche reveals integrin binding sialoprotein as a mediator of tumor cell migration. *Cell Rep.* **41**, 111511 https://doi.org/10.1016/j.celrep.2022.111511

25 Kurihara-Shimomura, M., Sasahira, T., Shimomura, H. and Kirita, T. (2020) Peroxidan plays a tumor-promoting role in oral squamous cell carcinoma. *Int. J. Mol. Sci.* **21**, 5416 https://doi.org/10.3390/ijms21155416

26 Li, W., Zhang, Q., Wang, X., Wang, H., Zuo, W., Xie, H. et al. (2021) Comparative proteomic analysis to investigate the pathogenesis of oral adenoid cystic carcinoma. *ACS Omega* **6**, 18623–18634 https://doi.org/10.1021/acsomega.1c01270

27 Dougan, J., Hawsawi, O., Burton, L.J., Edwards, G., Jones, K., Zou, J. et al. (2019) Proteomics-metabolomics combined approach identifies peroxidasin as a protector against metabolic and oxidative stress in prostate cancer. *Int. J. Mol. Sci.* **20**, 3046 https://doi.org/10.3390/ijms20123046

28 Zheng, Y.-Z. and Liang, L. (2018) High expression of PXDN is associated with poor prognosis and promotes proliferation, invasion as well as migration in ovarian cancer. *Ann. Diagn. Pathol.* **34**, 161–165 https://doi.org/10.1016/j.anndiagpath.2018.03.002

29 Ding, J., Liu, Y. and Lai, Y. (2020) Identifying MMP14 and COL12A1 as a potential combination of prognostic biomarkers in pancreatic ductal adenocarcinoma using integrated bioinformatics analysis. *PeerJ* **8**, e10419 https://doi.org/10.7717/peerj.10419

30 Li, C., Dong, X., Yuan, Q., Xu, G., Di, Z., Yang, Y. et al. (2023) Identification of novel characteristic biomarkers and immune infiltration profile for the anaplastic thyroid cancer via machine learning algorithms. *J. Endocrinol. Invest.* **46**, 1633–1650 https://doi.org/10.1007/s40618-023-02022-6

31 Di, Y., Chen, D., Yu, W. and Yan, L. (2019) Bladder cancer stage-associated hub genes revealed by WGCNA co-expression network analysis. *Hereditas* **156**, 7 https://doi.org/10.1186/s41065-019-0083-y

32 Chen, Z., Jiang, W., Li, Z., Zong, Y. and Deng, G. (2022) Immune-and metabolism-associated molecular classification of ovarian cancer. *Front. Oncol.* **12**, 877369 https://doi.org/10.3389/fonc.2022.877369

33 Wu, J., Wang, X., Wang, N., Ma, L., Xie, X., Zhang, H. et al. (2021) Identification of novel antioxidant gene signature to predict the prognosis of patients with gastric cancer. *World J. Surg. Oncol.* **19**, 219 https://doi.org/10.1186/s12957-021-02328-w

34 Gao, J., Zhao, Z., Zhang, H., Huang, S., Xu, M. and Pan, H. (2022) Transcriptomic characterization and construction of M2 macrophage-related prognostic and immunotherapeutic signature in ovarian metastasis of gastric cancer. *Cancer Immunol. Immunother.* **72**, 1121–1138 https://doi.org/10.1007/s00262-022-03316-z

35 Fang, F., Wang, P., Huang, H., Ye, M., Liu, X. and Li, Q. (2022) M6a RNA methylation regulator-based signature for prognostic prediction and its potential immunological role in uterine corpus endometrial carcinoma. *BMC Cancer* **22**, 1364 https://doi.org/10.1186/s12885-022-10490-x

36 Quan, K., Cao, Z., Xu, Q., Zhu, M., Liu, X. and Dai, M. (2022) A web-based calculator for predicting the prognosis of patients with sarcoma on the basis of antioxidant gene signatures. *Aging (Albany, NY)* **14**, 1407–1428 https://doi.org/10.18632/aging.203885

37 Sigurdardottir, A.K., Jonasdottir, A.S., Asbjarnarson, A., Helgudottir, H.R., Gudjonsson, T. and Traustadottir, G.A. (2021) Peroxidasin enhances basal phenotype and inhibits branching morphogenesis in breast epithelial progenitor cell line D492. *J. Mammary Gland Biol. Neoplasia* **26**, 321–338 https://doi.org/10.1007/s10911-021-09507-1

38 Pitteri, S.J., Kelly-Spratt, K.S., Gurley, K.E., Kennedy, J., Buson, T.B., Chin, A. et al. (2011) Tumor microenvironment-derived proteins dominate the plasma proteome response during breast cancer induction and progression. *Cancer Res.* **71**, 5090–5100 https://doi.org/10.1158/0008-5472.CAN-11-0568

39 Tang, Z., Li, C., Kang, B., Gao, G., Li, C. and Zhang, Z. (2017) GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res.* **45**, W98–W102 https://doi.org/10.1093/nar/gkx247

40 Yan, X., Sabrautzki, S., Horsch, M., Fuchs, H., Gailus-Durner, V., Beckers, J. et al. (2014) Peroxidasin is essential for eye development in the mouse. *Hum. Mol. Genet.* **23**, 5597–5614 https://doi.org/10.1093/hmg/ddu274

41 Cao, J., Zhang, G., Liu, Z., Xu, Q., Li, C., Cheng, G. et al. (2021) Peroxidasin promotes diabetic vascular endothelial dysfunction induced by advanced glycation end products via NOX2/HOCl/Akt/eNOS pathway. *Redox Biol.* **45**, 102031 https://doi.org/10.1016/j.redox.2021.102031

42 Barnett, P., Arnold, R.S., Mezencev, R., Chung, L.W.K., Zayzafoon, M. and Odero-Marah, V. (2011) Snail-mediated regulation of reactive oxygen species in ARCaP human prostate cancer cells. *Biochem. Biophys. Res. Commun.* **404**, 34–39 https://doi.org/10.1016/j.bbrc.2010.11.044

43 Sitole, B.N. and Mavri-Damelin, D. (2018) Peroxidasin is regulated by the epithelial-mesenchymal transition master transcription factor Snai1. *Gene* **646**, 195–202 https://doi.org/10.1016/j.gene.2018.01.011

44 Kadioglu, O., Saeed, M.E.M., Mahmoud, N., Azawi, S., Mrasek, K., Liehr, T. et al. (2021) Identification of novel drug resistance mechanisms by genomic and transcriptomic profiling of glioblastoma cells with mutation-activated EGFR. *Life Sci.* **284**, 119601 https://doi.org/10.1016/j.lfs.2021.119601

45 Tauber, S., Jais, A., Jeitler, M., Haider, S., Husa, J., Lindroos, J. et al. (2010) Transcriptome analysis of human cancer reveals a functional role of heme oxygenase-1 in tumor cell adhesion. *Mol. Cancer.* **9**, 200 https://doi.org/10.1186/1476-4598-9-200

46 Péterfi, Z., Donkó, A., Orient, A., Sum, A., Prókai, A., Molnár, B. et al. (2009) Peroxidasin is secreted and incorporated into the extracellular matrix of myofibroblasts and fibrotic kidney. *Am. J. Pathol.* **175**, 725–735 https://doi.org/10.2353/ajpath.2009.080693

47 George, J.T., Jolly, M.K., Xu, S., Somarelli, J.A. and Levine, H. (2017) Survival outcomes in cancer patients predicted by a partial EMT gene expression scoring metric. *Cancer Res.* **77**, 6415–6428 https://doi.org/10.1158/0008-5472.CAN-16-3521

48 Bergman, D.R., Karikomi, M.K., Yu, M., Nie, Q. and MacLean, A.L. (2021) Modeling the effects of EMT-immune dynamics on carcinoma disease progression. *Commun. Biol.* **4**, 983 https://doi.org/10.1038/s42003-021-02499-y

49 Wang, Q., Karvelsson, S.T., Kotronoulas, A., Gudjonsson, T., Halldorsson, S. and Rolfsson, O. (2022) Glutamine-fructose-6-phosphate transaminase 2 (GFPT2) Is upregulated in breast epithelial-mesenchymal transition and responds to oxidative stress. *Mol. Cell. Proteomics* **21**, 100185 https://doi.org/10.1016/j.mcpro.2021.100185

50 Chang, J. and Chaudhuri, O. (2019) Beyond proteases: basement membrane mechanics and cancer invasion. *J. Cell Biol.* **218**, 2456–2469 https://doi.org/10.1083/jcb.201903066

51 Kalluri, R. (2003) Basement membranes: structure, assembly and role in tumour angiogenesis. *Nat. Rev. Cancer* **3**, 422–433 https://doi.org/10.1038/nrc1094

52 Banerjee, S., Lo, W.-C., Majumder, P., Roy, D., Ghorai, M., Shaikh, N.K. et al. (2022) Multiple roles for basement membrane proteins in cancer progression and EMT. *Eur. J. Cell Biol.* **101**, 151220 https://doi.org/10.1016/j.ejcb.2022.151220

53 Mayorca-Guiliani, A.E., Madsen, C.D., Cox, T.R., Horton, E.R., Venning, F.A. and Erler, J.T. (2017) ISDot: *in situ* decellularization of tissues for high-resolution imaging and proteomic analysis of native extracellular matrix. *Nat. Med.* **23**, 890–898 https://doi.org/10.1038/nm.4352

54 Öhlund, D., Franklin, O., Lundberg, E., Lundin, C. and Sund, M. (2013) Type IV collagen stimulates pancreatic cancer cell proliferation, migration, and inhibits apoptosis through an autocrine loop. *BMC Cancer* **13**, 154 https://doi.org/10.1186/1471-2407-13-154

55 Wegner, C.S., Gaustad, J.-V., Andersen, L.M.K., Simonsen, T.G. and Rofstad, E.K. (2016) Diffusion-weighted and dynamic contrast-enhanced MRI of pancreatic adenocarcinoma xenografts: associations with tumor differentiation and collagen content. *J. Transl. Med.* **14**, 161 https://doi.org/10.1186/s12967-016-0920-y

56   Bai, Y.-P., Hu, C.-P., Yuan, Q., Peng, J., Shi, R.-Z., Yang, T.-L. et al. (2011) Role of VPO1, a newly identified heme-containing peroxidase, in ox-LDL induced endothelial cell apoptosis. *Free Radic. Biol. Med.* **51**, 1492–1500 https://doi.org/10.1016/j.freeradbiomed.2011.07.004

57   Tian, R., Yang, C., Chai, S.-M., Guo, H., Seim, I. and Yang, G. (2022) Evolutionary impacts of purine metabolism genes on mammalian oxidative stress adaptation. *Zool. Res.* **43**, 241–254 https://doi.org/10.24272/j.issn.2095-8137.2021.420

58   Sevcnikar, B., Paumann-Page, M., Hofbauer, S., Pfanzagl, V., Furtmüller, P.G. and Obinger, C. (2020) Reaction of human peroxidasin 1 compound I and compound II with one-electron donors. *Arch. Biochem. Biophys.* **681**, 108267 https://doi.org/10.1016/j.abb.2020.108267

59   Dempsey, B., Cruz, L.C., Mineiro, M.F., da Silva, R.P. and Meotti, F.C. (2022) Uric acid reacts with peroxidasin, decreases collagen IV crosslink, and impairs human endothelial cell migration and adhesion. *Antioxidants* **11**, 1117 https://doi.org/10.3390/antiox11061117

60   Li, C., Liu, Z., Xu, Q., Peng, H., Cao, J., Zhou, H. et al. (2021) PXDN reduces autophagic flux in insulin-resistant cardiomyocytes via modulating FoxO1. *Cell Death Dis.* **12**, 418 https://doi.org/10.1038/s41419-021-03699-4

61   Elliott, B.A., Ho, H.-T., Ranganathan, S.V., Vangaveti, S., Ilkayeva, O., Abou Assi, H. et al. (2019) Modification of messenger RNA by 2'-O-methylation regulates gene expression in vivo. *Nat. Commun.* **10**, 3401 https://doi.org/10.1038/s41467-019-11375-7

62   Luo, X., Zhou, Z., Wu, J., Zhang, L., Zhang, J. and Li, J. (2022) Integrated RNA- and miRNA-sequencing analysis identifies molecular basis for stress-induced heart injury in mouse models. *Int. J. Cardiol.* **349**, 115–122 https://doi.org/10.1016/j.ijcard.2021.11.084

63   Kovács, H.A., Lázár, E., Várady, G., Sirokmány, G. and Geiszt, M. (2021) Characterization of the proprotein convertase-Mediated processing of peroxidasin and peroxidasin-like protein. *Antioxidants (Basel)* **10**, 1565 https://doi.org/10.3390/antiox10101565

64   Colon, S. and Bhave, G. (2016) Proprotein convertase processing enhances peroxidasin activity to reinforce collagen IV. *J. Biol. Chem.* **291**, 24009–24016 https://doi.org/10.1074/jbc.M116.745935

65   Graf, F., Horn, P., Ho, A.D., Boutros, M. and Maercker, C. (2021) The extracellular matrix proteins type I collagen, type III collagen, fibronectin, and laminin 421 stimulate migration of cancer cells. *FASEB J.* **35**, e21692 https://doi.org/10.1096/fj.202002558RR

66   Wang, Z., Zhai, Z., Chen, C., Tian, X., Xing, Z., Xing, P. et al. (2022) Air pollution particles hijack peroxidasin to disrupt immunosurveillance and promote lung cancer. *eLife* **11**, e75345 https://doi.org/10.7554/eLife.75345

67   Yao, B., Zhu, S., Wei, X., Chen, M.-K., Feng, Y., Li, Z. et al. (2022) The circSPON2/miR-331-3p axis regulates PRMT5, an epigenetic regulator of CAMK2N1 transcription and prostate cancer progression. *Mol. Cancer* **21**, 119 https://doi.org/10.1186/s12943-022-01598-6

68   Ying, L., Sharma, A., Chhoda, A., Ruzgar, N., Hasan, N., Kwak, R. et al. (2021) Methylation-based cell-free DNA signature for early detection of pancreatic cancer. *Pancreas* **50**, 1267–1273 https://doi.org/10.1097/MPA.0000000000001919

69   Su, J.-Q., Lai, P.-Y., Hu, P.-H., Hu, J.-M., Chang, P.-K., Chen, C.-Y. et al. (2022) Differential DNA methylation analysis of SUMF2, ADAMTS5, and PXDN provides novel insights into colorectal cancer prognosis prediction in Taiwan. *World J. Gastroenterol.* **28**, 825–839 https://doi.org/10.3748/wjg.v28.i8.825

70   KrishnaPriya, S., Omer, S., Banerjee, S., Karunagaran, D. and Suraishkumar, G.K. (2022) An integrated approach to understand fluid shear stress-driven and reactive oxygen species-mediated metastasis of colon adenocarcinoma through mRNA-miRNA-lncRNA-circRNA networks. *Mol. Genet. Genomics* **297**, 1353–1370 https://doi.org/10.1007/s00438-022-01924-z

71   Hanmer, K.L. and Mavri-Damelin, D. (2018) Peroxidasin is a novel target of the redox-sensitive transcription factor Nrf2. *Gene* **674**, 104–114 https://doi.org/10.1016/j.gene.2018.06.076

72   Briem, E., Budkova, Z., Sigurdardottir, A.K., Hilmarsdottir, B., Kricker, J., Timp, W. et al. (2019) MiR-203a is differentially expressed during branching morphogenesis and EMT in breast progenitor cells and is a repressor of peroxidasin. *Mech. Dev.* **155**, 34–47 https://doi.org/10.1016/j.mod.2018.11.002

73   Zhang, Y., Zhang, R., Ding, X. and Ai, K. (2018) EFNB2 acts as the target of miR-557 to facilitate cell proliferation, migration and invasion in pancreatic ductal adenocarcinoma by bioinformatics analysis and verification. *Am. J. Transl. Res.* **10**, 3514–3528 ISSN:1943-8141/AJTR0084066

74   Hastings, J.F., Skhinas, J.N., Fey, D., Croucher, D.R. and Cox, T.R. (2019) The extracellular matrix as a key regulator of intracellular signalling networks. *Br. J. Pharmacol.* **176**, 82–92 https://doi.org/10.1111/bph.14195

75   Lee, S.-W., Kim, H.-K., Naidansuren, P., Ham, K.A., Choi, H.S., Ahn, H.-Y. et al. (2020) Peroxidasin is essential for endothelial cell survival and growth signaling by sulfilimine crosslink-dependent matrix assembly. *FASEB J.* **34**, 10228–10241 https://doi.org/10.1096/fj.201902899R

76   Young, C.D., Zimmerman, L.J., Hoshino, D., Formisano, L., Hanker, A.B., Gatza, M.L. et al. (2015) Activating PIK3CA mutations induce an epidermal growth factor receptor (EGFR)/extracellular signal-regulated kinase (ERK) paracrine signaling axis in basal-like breast cancer. *Mol. Cell. Proteomics* **14**, 1959–1976 https://doi.org/10.1074/mcp.M115.049783

77   Li, T., Liu, B., Li, N.-S., Luo, X.-J., Peng, J.-W. and Peng, J. (2022) Vascular peroxidase 1 promotes phenotypic transformation of pulmonary artery smooth muscle cells via ERK pathway in hypoxia-induced pulmonary hypertensive rats. *Life Sci.* **307**, 120910 https://doi.org/10.1016/j.lfs.2022.120910

78   Peng, H., Zhang, K., Liu, Z., Xu, Q., You, B., Li, C. et al. (2018) VPO1 modulates vascular smooth muscle cell phenotypic switch by activating extracellular signal-regulated kinase 1/2 (ERK 1/2) in abdominal aortic aneurysms. *J. Am. Heart Assoc.* **7**, e010069 https://doi.org/10.1161/JAHA.118.010069

79   Fatherree, J.P., Guarin, J.R., McGinn, R.A., Naber, S.P. and Oudin, M.J. (2022) Chemotherapy-induced collagen IV drives cancer cell motility through activation of Src and focal adhesion kinase. *Cancer Res.* **82**, 2031–2044 https://doi.org/10.1158/0008-5472.CAN-21-1823

80   Yang, Y., Shi, R., Cao, Z., Zhang, G. and Cheng, G. (2016) VPO1 mediates oxidation of LDL and formation of foam cells. *Oncotarget* **7**, 35500–35511 https://doi.org/10.18632/oncotarget.9193

81   Pape, V.F.S., Kovács, H.A., Szatmári, I., Ugrai, I., Szikora, B., Kacskovics, I. et al. (2022) Measuring peroxidasin activity in live cells using bromide addition for signal amplification. *Redox Biol.* **54**, 102385 https://doi.org/10.1016/j.redox.2022.102385

82   Colon, S., Luan, H., Liu, Y., Meyer, C., Gewin, L. and Bhave, G. (2019) Peroxidasin and eosinophil peroxidase, but not myeloperoxidase, contribute to renal fibrosis in the murine unilateral ureteral obstruction model. *Am. J. Physiol. Renal Physiol.* **316**, F360–F371 https://doi.org/10.1152/ajprenal.00291.2018

83   The Role of Peroxidasin in Basement Membrane Physiology and Human Disease - ProQuest [Internet]. [cited 2023 Apr 4]. Available from: https://www.proquest.com/docview/1845871387?pq-origsite=gscholar&fromopenview=true

84   Panagopoulos, V., Leach, D.A., Zinonos, I., Ponomarev, V., Licari, G., Liapis, V. et al. (2017) Inflammatory peroxidases promote breast cancer progression in mice via regulation of the tumour microenvironment. *Int. J. Oncol.* **50**, 1191–1200 https://doi.org/10.3892/ijo.2017.3883

85 Cappelletti, P., Doretto, P., Signori, D. and Bizzaro, N. (1992) Eosinophilic peroxidase deficiency. cytochemical and ultrastructural characterization of 21 new cases. *Am. J. Clin. Pathol.* **98**, 615–622 https://doi.org/10.1093/ajcp/98.6.615

86 Pahwa, R., Modi, P. and Jialal, I. (2023) *Myeloperoxidase Deficiency. StatPearls*, StatPearls Publishing, Treasure Island, FL

87 DeNichilo, M.O., Panagopoulos, V., Rayner, T.E., Borowicz, R.A., Greenwood, J.E. and Evdokiou, A. (2015) Peroxidase enzymes regulate collagen extracellular matrix biosynthesis. *Am. J. Pathol.* **185**, 1372–1384 https://doi.org/10.1016/j.ajpath.2015.01.013

88 Kim, H.-K., Ham, K.A., Lee, S.-W., Choi, H.S., Kim, H.-S., Kim, H.K. et al. (2019) Biallelic deletion of pxdn in mice leads to anophthalmia and severe eye malformation. *Int. J. Mol. Sci.* **20**, 6144 https://doi.org/10.3390/ijms20246144