

NUPR1 and its potential role in cancer and pathological conditions (Review)

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Abstract. Nuclear protein-1 (NUPR1) is also known as Com-1 or p8. It is a protein primarily found in the nucleus of various cells, including cancer cells, and it has been found to play an important role in cell stress and stress-related apoptosis. Over the past two decades, NUPR1 has been firmly indicated to play a role in the development and progression of numerous types of cancer, as well as in a number of other pathological conditions, including pancreatitis, diabetes, neurological and inflammatory conditions. The past decade has witnessed a rapid understanding of the biological and cellular mechanisms through which NUPR1 operates on cells and the identification of new variant of the protein. Most importantly, there have been comprehensive studies on the clinical and pathological aspects of NUPR1 and its variant in multiple malignancies and identification of therapeutic methods by targeting the protein. The present review aimed to summarise the current knowledge relating to NUPR1 in human malignancies and to discuss the associated controversies and potential future prospects of this molecule.

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1. Introduction

Nuclear protein-1 (NUPR1; also known as Com-1 or p8) was initially discovered in 1997 and expressed as a small protein in the rat and as a novel gene that is activated in the acute phase of induced pancreatitis and during pancreatic development (1). The molecule, then named p8 was shown to be related to cell death and gene transcription. During the following 2 years, the same study group discovered the human version of p8, which shares a 74% similarity with rat p8 (1,2). Independently, in the same year, a second group discovered a new molecule in their search for candidate gene(s) associated with brain metastasis of breast cancer (3). In metastatic brain tumours, the expression of one gene was found to be elevated, compared with the parent breast cancer, and was named candidate of metastasis-1 or Com-1. It was soon found that these molecules were identical. In 2003, Quirk *et al* isolated a gene clone from pituitary derived cells and found it to encode p8 (4). These findings have since triggered active research into the role of this protein in cancer and other pathological conditions, such as pancreatitis. NUPR-2 or NUPR1-like protein was then identified (5), which appears antagonistic to some degree, to the function of NUPR1. Together, NUPRs indicate a fascinating area of research. The present review aimed to summarise the progress in studies on NUPR1, primarily in cancer and to a limited degree, in other pathological conditions.

2. Cellular function of NUPR1

There are some excellent reviews available in the literature which provide detailed knowledge of the molecular and cellular function of NUPR1 at the beginning of this decade (6,7). The present review describes the advancements made in the understanding of NUPR1 in various cancer types, and some benign disorders, which have taken place since, and summarizes the key functions of NUPR1 in cells, as well as associated genetic interactions, which are illustrated in Fig. 1.

Transcriptional regulations of NUPR1. NUPR1 is a transcription regulator protein with its gene located on chromosome 16. It is typically expressed in response to stress signals induced by genotoxic signals and agents. Transforming growth factor β (TGF β) is an important regulator of NUPR1 transcription. TGF β , upon binding to its receptor, initiates a canonical

cascade, in which phosphorylated small mothers against decapentaplegic (SMAD)-2/3 proteins form a heteromeric complex with cofactor SMAD-4 and translocates into the nucleus. By binding on to the promotor at the 5'-untranslated region (5'-UTR), it elevates the transcription of NUPR1 through binding, a regulation appearing at a rapid pace (8).

DNA damage and repair. NUPR1 influences cancer cell resistance to metabolic stress-induced glucose starvation and hypoxia through the downstream regulation of Aurora kinase A (AURKA) expression. The inhibition of AURKA triggers a cytotoxin that can lead to DNA damage (9). NUPR1 is also important in γ -irradiation-induced damage and repair (10). It negatively controls DNA repair following γ -irradiation in the presence of MSL complex subunit 1 (MSL1) by regulating histone acetyltransferase (HAT) activity and is involved in intercommunication with p53 binding protein (P53BP1) (10). It has also been shown that the inhibition of NUPR1 by the organic synthetic molecule, ZZW-115, sensitizes cells to DNA damage, resulting in the reduction of SUMOylation of several proteins involved in DNA damage response by inhibiting the nuclear translocation of NUPR1. This decreases the SUMOylation-dependent functions of proteins involved in the DNA damage response (6).

Cell stress and cell death. NUPR1 regulates cellular damage and death in different forms, depending on the cell context and the types of stress induced. NUPR1 is involved in D9-tetrahydrocannabinol (THC)-induced cancer cell death through the downstream targeting of death inducible telomere repeat-binding factor 3 (TRB3) protein, transcription factor activating transcription factor 4 (ATF-4) and the protein C/EBP homologous protein (CHOP) following endoplasmic reticulum stress (ERS) elevated from the synthesis of ceramide and collectively provokes apoptosis (11). NUPR1 downregulation in hepatocellular carcinoma (HCC) cells can enhance cell sensitivity to sorafenib treatment, which further controls cell growth through the RELB/IER3 pathway (12). The previous study by Santofimia-Castaño *et al.* (2018) demonstrated that cell death observed following the knockdown of NUPR1 expression could be reversed by incubation with necrostatin-1, but not by the inhibition of caspase activity (13). The authors of that study thus described a model in which inactivation of NUPR1 in pancreatic cancer cells resulted in ERS that induced a mitochondrial malfunction, a deficient ATP production and, as consequence, cell death mediated by a programmed necrosis (13).

Cell growth, autophagy and death. NUPR1 promotes the proliferation of cancer cells by influencing cell cycle progression. NUPR1 can aid cells to enter the S phase by bypassing the G0/G1 checkpoint. Escape from the G0/G1 phase is achieved by an association between NUPR1 and cyclin inhibitory proteins resulting in downregulation of p21 and p57 (14). The knockdown of NUPR1 is able to regulate autophagy by interfering with FoxO3, promoting Bnip3 transcription in the control of autophagy, a stress-dependent self-defence mechanism that helps cells eliminate the toxic microenvironment (15). It has been reported that NUPR1 silencing suppresses autophagic activities and induces

autophagy-mediated apoptosis in multiple myeloma (MM) cells through the PI3K/AKT/mammalian target of rapamycin (mTOR) pathway, which exhibits potential as a treatment strategy for MM (16), and that NUPR1 is a potent regulator of autolysosomal dynamics and is required for the progression of certain epithelial cancers as it regulates the late stages of autolysosome processing through the induction of the synaptosomal-associated protein (SNAP)-receptor (SNARE) protein synaptosomal-associated protein, 25 kDa (SNAP25), which forms a complex with the lysosomal SNARE-associated protein, VAMP8. NUPR1 depletion deregulates autophagic flux and impairs autolysosomal clearance, inducing massive cytoplasmic vacuolization and premature senescence *in vitro* and tumour suppression *in vivo* (17).

Cell senescence. It has been found that in disordered pancreatic mouse cells, the inhibition of NUPR1 facilitates Kras-induced cellular senescence through the genomic downregulation of Dnmt1 expression, an enzyme transferring methyl groups onto DNA, which in turn decreases DNA methylation, crucial for transformation that helps the induction of Kras-dependent pancreatic cancer (18). NUPR1 can directly regulate the expression of DNA (cytosine-5)-methyltransferase 1 (Dnmt1) by interfering with its transcription process by binding on to the promoter (19). The silencing of NUPR1 promotes stress-induced senescence upon the enlarging flattened phenotype of cells provoking spatial pressure (14). As discussed above, NUPR1 is aberrantly expressed in a subset of cancer cells and predicts low overall survival rates for patients with lung cancer. NUPR1 depletion deregulates autophagic flux and impairs autolysosomal clearance, inducing massive cytoplasmic vacuolization and premature senescence (17).

Endothelial cells. As previously discussed by Cai *et al.* (2016) in the context of methamphetamine (METH)-induced endothelial apoptosis, NUPR1 functions as a fundamental regulator throughout the whole process (20). METH-associated disruptive effects give rise to the formation of ERS for compensating cell damage in endothelial cells. NUPR1 elevates transcription factor CHOP production in response to ERS, which then couples to the PUMA promoter through the mediation of p53, a downstream protein after the nupr1/chop axis. The succeeding cascades are achieved based on the presentation of NUPR1. The action of CHOP dissociates anti-apoptotic BCL-2 and upregulates pro-apoptotic BAX, as well as altering the membrane potential of the mitochondria. The resulting increased BAX/Bcl-2 ratio drives the apoptogenic factor cytochrome *c* importing from mitochondria into the cell cytosol, which then successfully triggers endothelial caspase-mediated cell death. A previous study by Tang *et al.* (2015) also revealed the importance of a reciprocal association between NUPR1 and ER stress by investing in *Shigella* enterotoxin (Shiga) toxin-induced enterocyte apoptosis (21).

Influence on metabolism. In 2018, Santofimia-Castaño *et al.* examined NUPR1 depletion cooperating with ERS, inducing pancreatic cancer cell apoptosis and programmed necrosis by mediating cellular metabolism. Following NUPR1 knockdown, there was a decrease in ATP production, resulting in deficient oxygen availability (13). Mitochondrial membrane

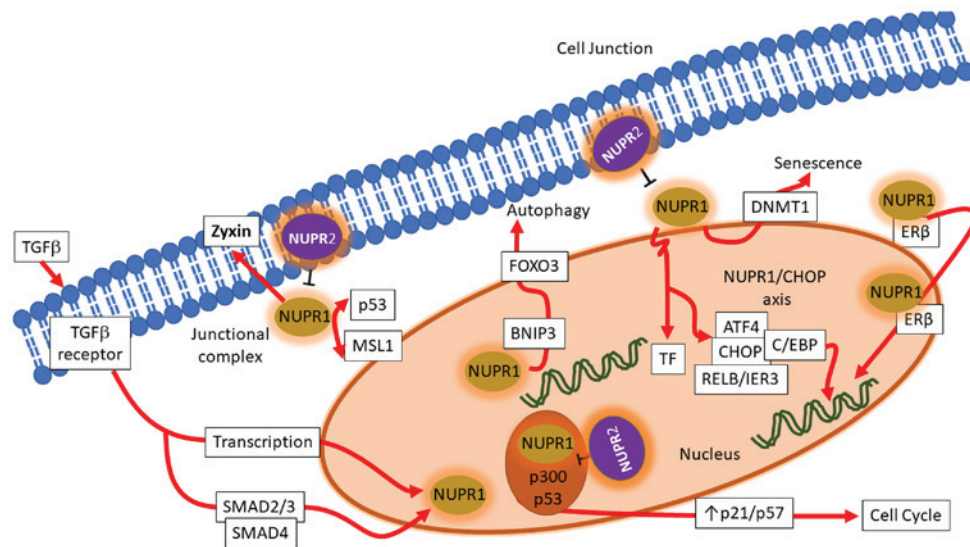


Figure 1. Schematic summarizing the key functions and interactions of NUPR1 in cells. NUPR1, nuclear protein-1. NUPR2 has been shown to block the activity of NUPR1 where indicated in the diagram.

potential disruption following Ca^{2+} uptake from the cytoplasm is also initiated by a shortage of NUPR1 together with ERS. The updated mitochondria content triggers the alteration in membrane permeability resulting in the discharge of cytochrome *c*, which leads to cell death (20). Other research has elucidated a confirmed association between the deficiency of NUPR1 and bone metabolism, by mediating the receptor activator of nuclear factor kappa-B ligand or (RANK ligand or RANKL) and sclerostin, which in turn enhances the proliferation of osteoblasts and the downregulation of osteoclasts (22). NUPR1 interacts with and activates SNAP25 to initiate an autolysosomal process that results in premature senescence and autophagy process (17). NUPR1 is an essential regulator in protein metabolism and glucose homeostasis (23). It interacts with p300 and Pax2 through which it regulates the transactivation activity of Pax2A and Pax2B and influences the promoter activities of the glucagon gene (24). It is also a mediator of glucose induced growth of beta cells in the pancreas (25). It is also downstream of Zyxin, an adhesion junctional regulator protein with a controversial role in cancer (26).

3. NUPR1 in malignant disease

Overall involvement in cancer. Investigations into the role of NUPR1 have increased since the discovery that it was linked to brain metastasis in breast cancer (3). By injecting the human breast cancer cell line, MA-11, into athymic rats, brain metastases were established. In comparisons between the primary cancer cells and metastatic breast cancers of the brain using differential RNA display and protein analysis, NUPR1 was found to be present in metastatic cells and not in primary cancer cells and was found to be in aggressive MDA MB-231, but not in MCF-7 cells which are less aggressive (3). The authors of that study also demonstrated that there was a rapid rise in establishing a clinical link between NUPR1 and the development, progression and clinical outcome in various types of cancer. The tumorigenic effect of NUPR1

was subsequently demonstrated in that embryonic fibroblasts from NUPR1^{+/+} mice, when transformed with ras V12 mutation became tumorigenic and spread into the peritoneal cavity of mice compared with the same transformation of NUPR1^{-/-} fibroblasts, which were non-tumorigenic (27,28). It was surprising to note that NUPR1^{+/+} cells with ras V12 mutation transformation grew at a slower rate than the NUPR1^{-/-} cells with ras V12 mutation transformation, in clear contrast to the *in vivo* results in the same study (28). Another observation made by the same authors was that the NUPR1-deficient cells grew more rapidly than the NUPR1^{+/+} cells (27). The clear discrepancy and disconnection between *in vitro* cell growth and *in vivo* tumour growth remain unexplained.

However, these early observations have driven a marked interest in examining the role of the molecule in individual human cancers. A summary of some of the key findings from previous studies is presented in Table I.

Breast cancer. In breast cancer cells, NUPR1 has been shown to form a complex with p300 and p53 together with the p21 promoter, to upregulate the expression of p21, allowing breast cancer cell to progress through the cell cycle (29). In MCF-7 breast cancer cells, a HSP9 chaperone protein p23 was shown to result in 7.6 fold downregulation, although the response to oestradiol was less prominent (30). It has been reported that breast cancer cells have less nuclear staining than normal cells, but more cytoplasmic staining in mammary tumour tissues (31). A study on gene transcripts also demonstrated that NUPR1 was reduced in aggressive tumours and that high levels of NUPR1 transcript were associated with a longer survival (31). The finding that NUPR1 levels in oestrogen receptor (ER) and ERβ-negative tumours has an important bearing in survival led us to a further discovery that NUPR1 plays an interactive role with ERβ and that 17-β-oestradiol is able to impact the cellular location of NUPR1 in breast cancer cells (32). Using genetic analysis to detect copy numbers of NUPR1 and ERBB2 (*her2*), Jung *et al* (2012) found that a gain of copy numbers of both genes in early breast cancer

Table I. NUPR1 in clinical cancers.

Cancer type	Methods applied	Clinical relevance	(Refs.)
Breast cancer	Northern blot analysis (n=81)	High levels in tumours. No correlations with clinical and pathological parameters nor with uPA (urokinase-type plasminogen activator) and uPAR (urokinase-type plasminogen activator receptor)	(100)
	IHC and transcript analysis (n=120)	Reduced nucleus and increased cytoplasmic staining in cancer cells. High level is linked to good prognosis	(32)
	Genetic analysis of early stage breast cancer (n=145)	Simultaneous gain of NUPR1 and ERBB2 (receptor tyrosine- <i>protein</i> kinase erbB2 precursor) gene copy number indicate poorer clinical outcome	(33)
	Gene transcript by PCR (n=96)	Stepwise increase of NUPR1 mRNA from normal, stage 1, 2, 3 and 4.	(36)
Pancreatic cancer	IHC and PCR (38 pancreatic cancer, 5 liver metastasis and 7 metastatic lymph nodes)	Tumour tissues stained positive for p8 and normal tissue mostly negative.	(43)
	IHC (n=44)	Highly positive in nodal positive tumours and is inversely correlated with the presence of apoptotic cells. No correlation with survival.	(42)
	IHC on pancreatic ductal adenocarcinoma (n=34, TMA)	Level of the NUPR1 expression, together with hypoxia inducible factor 1 subunit α (HIF1 α) are inversely correlated with survival time	(9,44)
Colorectal cancer	IHC (n=36)	NUPR1 is linked with cannibalism of pancreatic cancer which in turn linked to prognosis	(101)
	IHC and quantitative gene transcript analysis (n=80)	Tumour tissues had higher levels of NUPR1 transcript than normal tissues. High stage tumours had less NUPR1. NUPR1 protein was more visible in nucleus in normal epithelial and in tumour cells stronger staining in the cytoplasm	(38)
Cholangio-carcinoma	NUPR1 transcript analysis by PCR (n=50)	NUPR1 mRNA was highly raised in tumour tissues than normal tissues	(39)
	IHC (n=10)	NUPR1 mostly nuclear staining in ductal epithelial cells, increased nuclear staining in cholangiocarcinoma cells	(60)
Prostate cancer	IHC	Reduced nucleus and cytoplasmic staining in prostate cancer cells, compared with normal prostate epithelial cells	(49)
Bladder cancer	IHC (n=37)	Mainly cytoplasmic staining, with invasive cancer cells stained less intensively	(50)
Lung cancer	Quantitative gene transcript analysis	High levels of NUPR1 mRNA in adenocarcinoma, squamous cell carcinoma and adenosquamous carcinoma compared with the adjacent normal tissues	(52)
	IHC (n=118), NSCLC (non small cell lung cancer)	High level staining of NUPR1 in cancer tissues and high staining associated with shorter survival.	(17)
Endometrial cancer	IHC (n=198) and gene transcript analysis (n=32)	High levels of NUPR1 protein and mRNA in deep tumours compared with superficial tumours	(57)
Multiple myeloma (MM)	Geodata analysis (n=152)		(58)
	Bone marrow from MM (N=4) and normal	High levels of NUPR1 mRNA in MM than in normal	(59)

Table I. Continued.

Cancer type	Methods applied	Clinical relevance	(Refs.)
Hepatocellular carcinoma (HCC)	Gene transcript analysis (N=23)	A portion of the tumours (4 out of 23) has high level	(62)
	IHC and gene array (n=35 including normal liver, non-tumour and tumour tissues)	Increase in NUPR1 staining in liver cancer (strong in nucleus and also with cytoplasmic staining). Transcription ratio (tumour to normal) 1.667 (P<0.005)	(63)
	HCC (n=21), cirrhotic liver (n=3) and normal liver (n=3) by IHC and qPCR. NUPR1 transcript analysis (n=158)	HCC with high levels of nuclear staining of NUPR1 and NUPR1 mRNA than normal liver tissues Significantly higher in HCC than normal liver tissues and in tumours with high vascular invasion. Combined expression of NUPR1 and thyroxin receptors significantly correlated with the survival of the patients	(12) (66)
Thyroid cancer	IHC (n=150)	Most normal and tumour tissues positive for staining and tumour tissues tended to be over-expressed. Anaplastic type less intense in staining than papillary and follicular types. Large tumours and those with lymph node involvement more intense.	(47)
	IHC (n=30) medullary carcinoma	43.4% regarded as highly expressed and high degree of staining linked to lymph node metastasis and recurrence	(48)
Glioma	IHC and QPCR (n=122)	High levels of NUPR1 mRNA seen in glioma tissues than normal tissues. High levels staining is associated with shorter survival of the patients.	(68)
Osteosarcoma	QPCR (n=58)	Osteosarcomas have significantly high levels of NUPR1 transcript than non-tumour tissues.	(67)

IHC, immunohistochemistry; qPCR, quantitative polymerase chain reaction; mRNA, messenger ribonucleic acid; PCR, polymerase chain reaction.

was a valuable predictor for patients with early-stage breast cancer (33). In animal models, NUPR1 together with BMP4, Cyr6, plod2 and angiotensin2, were shown to be markedly downregulated in transcription factor E2F knockdown mice and that collectively, these reductions were thought to contribute to the retardation of metastasis and presence of circulating cancer cells (34).

A more recent study has revealed that NUPR1 was essential for tumour repopulating cells in breast cancer cells (MCF-7) to grow, forming colonies and tumours *in vivo* (35). That study demonstrated that NUPR1 overexpression suppressed nestin and human telomerase reverse transcriptase (hTERT), both clonogenic markers, via the p53 pathway, leading to the inhibition of repopulation by this small population of cancer cells and a reduction in tumour growth *in vivo*, together revealing a tumour suppressor role for NUPR1 in breast cancer and indeed ovarian cancer (35). NUPR1 has been found to be present at high levels in breast cancer cells metastasised to bone, although not those to the brain, when compared with the parent cells (36). This is an

interesting finding and that, together with a recent study demonstrating that NUPR1 may be linked to the osteoclastic activities of bone (22), may suggest that NUPR1 plays a pivotal role in bone metastasis from breast cancer. This possibility is strengthened by findings that NUPR1 is also an important factor in the growth of bone marrow mesenchymal cells (37).

Colorectal cancer. Colorectal tumour tissues exhibit high levels of the NUPR1 transcript (38,39) and more cytoplasmic NUPR1 staining, compared with normal tissues (38). High stage tumours exhibited a less obvious presence of NUPR1. The same team demonstrated that the knockdown of NUPR1 from colorectal cancer cells (RKO and CaCo2) resulted in less growth, less colony formation and increased apoptosis, with minimal roles played in cellular migration, presenting a somewhat contrasting role to the clinical findings (39,40); Wang *et al* demonstrated that the knockdown of NUPR1 reduced invasiveness and that the overexpression of NUPR1 exerted opposite effects (39). It appears the NUPR1-like

protein (NUPR2 and NUPR11) plays a contrasting role to NUPR1 in colorectal cancer cells since the suppression of NUPR11 by miR2277 results in an increase in cell migration and cell growth (41).

Pancreatic cancer. Early reports of the clinical significance of NUPR1 in pancreatic cancer came from Su *et al.* (42,43), which demonstrated that pancreatic cancers, particularly metastatic and node-positive tumours exhibited high levels of NUPR1 protein, although no association with survival was established. A very compelling investigation revealed that the staining of NUPR1 protein in pancreatic ductal adenocarcinoma was inversely associated with the clinical outcome of patients over a 24-month follow-up period (44). That study also revealed that NUPR1, together with RelB and IER3, formed a group of markers not only for predicting patient outcome, but also in the development of intraductal neoplasia of the pancreas (44). Furthermore, NUPR1, together with hypoxia-inducible factor α (HIF α) and AURKA participated in the regulation of pancreatic cancer cell autophagy response to hypoxia and glucose deprivation (9). The loss/deletion of NUPR1 would result in the malfunction of the mitochondria due to ERS, leading to cell death (13). The pancreatic cancer cell line, colo357, is amongst the most sensitive cell types to a marked increase in NUPR1 expression in the response to TGF β 1 (8). In pancreatic cancer, NUPR1 is intimately involved in homotypic cannibalism, or cell-in-cell, in that there is virtually no NUPR1. The cell-in-cell phenomenon can be enhanced by TGF β in PANC1 cells (45). Using an elegant Pdx1-cre; LSL-Kras^{G12D}; Ink4a/Arf^{fl/fl} (KIC) mouse model, Cano *et al.* (2014) demonstrated that a proficient NUPR1 expression resulted in the development of murine pancreatic ductal adenocarcinoma. However, the deletion of the NUPR1 gene in these mice, although causing substantial perinatal death due to NUPR1 and Ink4a/Arf inactivation, the surviving mice exhibited a prolonged survival and less pancreatic cancer-related deaths (46). Pancreatic cancer cells derived from NUPR1-proficient KIC mice displayed a high degree of stemness and anchorage-independent growth compared with NUPR1-deficient cells (46).

Thyroid cancer. Normal thyroid tissue tends to have a low degree of NUPR1 staining while thyroid tumours have high levels (47). Papillary and follicular tumours stain stronger than anaplastic tumours and normal tissues. Node positive tumours also stain stronger than node-negative tumours (47). One of the most interesting observations from the study is the cellular protein location. In normal follicles, NUPR1 is exclusively displayed as nuclear staining. The same nuclear staining is observed in follicular tumours. However, papillary tumours largely stain the cytoplasmic region, particularly in those large tumour and tumours with lymph node metastasis (47). The majority of medullary tumours stain strongly for NUPR1 and high levels are linked to lymph node metastasis and recurrence (48).

Urological cancers. In a limited study on prostate cancer, nuclear and cytoplasmic staining were found in normal prostate epithelial cells and both staining patterns were reduced in prostate cancer cells (49). The knockdown of NUPR1 in

PC3, DU145 and CAHPV10 prostate cancer cells resulted in an increase in the invasiveness of the cells in their response to an invasion inducer, hepatocyte growth factor (HGF) (49). Bladder transitional cells largely have cytoplasmic staining, with invasive cancer staining at a lower intensity (50). The knockdown of NUPR1 from bladder cancer cell lines (RT112 and EJ138) results in an increase in both cell growth and invasiveness. In multiple bladder cancer cell lines, CUPR1 is one of the few epigenetically upregulated non-CpG island genes, together with TIMP1, TNFRSF14, ITGB4 and downregulated genes including MMP11 and FGF18 (51).

Lung cancer. Non-small cell lung cancer (NSCLC) tissues exhibit markedly high levels of NUPR1 protein staining compared with adjacent normal tissues and those with high levels in tumours have a significantly shorter overall survival (28 months), a marked difference from those with low levels (80 months) (17). There is otherwise no significant association with tumour staging, smoking or age. Tumour tissues (adenocarcinoma, squamous cell carcinoma and mixed type) have significantly higher levels of NUPR1 transcript than their normal counterpart tissues (52). The same group have also shown that multiple human lung cancer cell lines, namely A549, SKME1, 95-D, NCI-H460, H1299, all highly express NUPR1 (52). In these lung cancer cells, knock-down of NUPR1 by siRNA results in the cells forming less colonies, becoming more apoptotic and forming less tumours in *in vivo* models (17,52). It is also a key bystander response gene in the lung cancer cell line, H1299, when irradiated (53) and a responsive gene that is downregulated following the knockdown of a mitochondrial protein c3orf1 (translocase of inner mitochondrial membrane domain-containing protein 1) from the lung cancer cell line, 95D (54). In this case, the reduction in NUPR1 expression was also linked to a change in the cell cycle and the reduction in cellular migration.

Skin cancers. In K14 Δ NLef1/K14L61Rac1 double-transgenic mice, an exclusive skin tumour type, sebaceous carcinoma-like tumours are prevalent and are characterised by aggressive growth and progression (55). A previous study identified NUPR1 as one of the few responsive genes contributing to tumour progression as the result of RAC1 knockdown. In melanoma, the BRAF inhibitor, encorafenib, increased the expression of ATF4, CHOP and NUPR1 and induced the expression of PUMA (56).

Gynaecological cancers. In endometrial cancer cells, NUPR1, together with Nidogen 1 was found to be a target gene of the ETV5 transcription factor which is key to myoendometrial invasion by cancer cells (57). Eliminating NUPR1 in endometrial cells minimises cellular migration induced by ETV5 overexpression. NUPR1 was also shown to be highly expressed in the deep (invasive) endometrial cancers of the patients (57).

Myeloma. Using available GEO databases, Di Martino *et al.* (2015) identified that the action of NUPR1 was one of the key mechanisms in myeloma progression and was connected with the downregulation of 6 genes, including *BNIP3*, *GINS1*,

GRAMD3, KIF11, SHCBP1 and *SPIN4* and the upregulation of the 2 genes, *ELMOD1* and *SLC16A6* (58). Bone marrow from multiple myeloma patients tends to have higher levels of NUPR1 transcript than healthy volunteers, although in that study the sample number was small (59). The silencing NUPR1 in myeloma cells also resulted in a reduction of cell proliferation and induction of apoptosis and cell cycle blockage (59).

Biliary cancers. In cholangiocarcinoma, NUPR1 is predominantly stained in the nucleus and more so in cancer cells (60). A human HuCCT1 cholangiocarcinoma cell was found to have a reduced rate of cell growth, and migration and invasiveness in response to EGF and serum, following NUPR1 knockdown by siRNA (60).

Hepatocellular carcinoma (HCC). TGF β 1 was able to markedly increase the expression of NUPR1 in the Hep1 hepatocellular carcinoma cell line (8). In a pathway search study, NUPR1 was found amongst the top down-regulated genes in non-viral HCCs, namely in alcohol consumption-related HCC (z ratio-3.0) and non-alcoholic fatty liver disease related HCC (z ratio-4.5) (61). HCC tissues tend to have high levels of NUPR1 protein, mostly in the nucleus, although no association was observed with TNM staging (12). Lee *et al* (2015) reported that NUPR1 was a key transcription regulator which leads to defective mitochondria-regulating genes in HCC (62). In this process, which is linked to metabolism of the cancer type, granulin is the key downstream effector protein of NUPR1. Knockdown of NUPR1 leads to a calcium signalling-dependent reduction of cellular invasion (62). Of note, the evaluation of NUPR1 mRNA expression in the limited clinical cohort only revealed a small portion of increase in NUPR1 transcript. NUPR1 can be activated by hepatitis X protein (HBx) via the HBx-Smad4 pathway, which results in the reduction of cell death and the induction of vasculogenic mimicry in HCC (63). The same study demonstrated that NUPR1 transcript was found to be significantly upregulated in HCC compared with normal liver tissues by a ratio of 1.667. The knockdown of NUPR1 resulted in HCCs that were less mobile, had lower invasiveness and were less tumorigenic *in vivo*. It further identified the NUPR1/RELB/IER3/RUNX2 pathway as key in these events (12). In a comprehensive search for the transcriptomic and histone modification profiles during the transition from non-alcoholic steatohepatitis to HCC, it was found that NUPR1 plays an important role in this complex network (64). NUPR1 can inhibit lysine acetyltransferase 8, which in turn influences one of the key generic alterations of gene patterns for the deacetylation of histone H4 Lysin 16 during the transition process. Serum from patients with chronic hepatitis B has been shown to be able to activate expression of NUPR1 in HCC cells (65). Thyroxin (T3) is a potent inducer of NUPR1 expression in HCC cell lines, by over 30-fold, an effect attributable to the transcriptional regulation of thyroxin receptor protein directly binding and activating the transcriptional response elements of the NUPR1 promoter (66). Clinically, NUPR1 is positively associated with thyroxin receptors and the high levels of

expression of both are significantly linked to shorter overall and disease-free survival (60).

Osteosarcoma. Osteosarcoma tissue has significantly higher levels of NUPR1 transcript compared with normal tissues (67). Together with miR4443, NUPR1 plays a regulatory role in a long non-coding RNA, FEZF1-AS1, induced cell growth, and the migration and invasiveness of osteosarcoma cells. lncRNA FAL1 and FEZF1-AS1 have been shown to require NUPR1 in their cancer inducing activities, acting respectively with miR637 and miR4443 (39,67).

Neurological tumours. In gliomas, the NUPR1 transcript level is significantly higher than in normal tissues (68). High-grade glioma stains more strongly than normal brain tissue and low-grade tumours: Again, high levels of staining are associated with a shorter survival and also indicates NUPR1 to be an independent prognostic indicator (68). The study further confirmed that the knockdown of NUPR1 in multiple glioblastoma cells resulted in a reduction of cell migration and proliferation, and cell cycle arrest at the G0/G1 phase (69), events appearing to be coordinated by intracellular signalling events involving ERK1/2, p38 MAPK and cleavage of caspase-3 and p27 (68).

Pituitary tumours. There are currently no studies available on human tumours as yet, at least to the best of our knowledge. NUPR1 expression is generally quiescent in the pituitary gland. However, an animal study conducted by Mohammad *et al* (2004) demonstrated that a parent cell of GH3 somatolactotrope genotype and a gonadotropic pituitary cell, LbT2 was tumorigenic in nude mice, whereas when NUPR1 expression was reduced in these cell lines, it lost its ability to form tumours *in vivo* (70). It was subsequently established that at least in LbT2 gonadotropic pituitary cells, NUPR1 allows the cell to avoid the G0/G1 phase of the cell cycle (14) and that NUPR1 is transcriptionally regulated by activating transcription factor 4 (ATF4), as in other cell types, such as HeLa cells (71,72). The GCN/ATF4 pathway appears to involve the amino acid response element (AARE) with the NUPR1 promoter (73).

4. NUPR1 in other conditions

Neurological disorders. NUPR1 is one of the responsive genes in the cerebral cortex, which is downregulated in response to oestradiol (E2) induction (74). NUPR1, together with a few other proteins appears to be a key responsive molecule in neural cells and tissues following a challenge with methamphetamine (75). Treatment with this substance results in an increase in NUPR1 expression, which is associated with an increase in apoptosis and autophagy in neural cells *in vivo* and in cell lines *in vitro* (68).

Cardiac hypertrophy. Cardiac hypertrophy is the abnormal enlargement, or thickening, of the heart muscle, resulting from increases in cardiomyocyte size. It has been reported that NUPR1 is an important factor in cardiomyocyte hypertrophy, induced by endothelin and phenylephrine (76). NUPR1 is also key to transforming growth factor α (TNF α)-induced

metalloproteinases in heart fibroblasts. These are key contributing factors in heart failure. It has also been observed that NUPR1 also partners with some of the muscle specific genes such as p68 (Ddx5) and MyoD in myoblasts (77).

Liver toxicity. NUPR1 is part of a protection mechanism in CCL4 [Chemokine (C-C motif) ligands 4] induced liver injury, by coordinating with cytochrome P450 2E1 which converts the chemical to toxic products (78).

Inflammation. The loss of NUPR1 in mice has been shown to result in increased death when challenged by lipopolysaccharide (LPS), together with an increase in TNF α , and in the reactive oxygen species, myeloperoxidase and hydroperoxide, suggesting that the loss of protection of stress injuries by NUPR1 (27,79). Conversely, TNF α via NF κ B mediates the expression of NUPR1 (80). It has also been indicated to be involved in the pathophysiological process of arthritis, in that osteoarthritic cartilage has higher levels of NUPR1 and that NUPR1 appears to be a key mediator in interleukin-1 β induced MMP13 expression in chondrocytes (81).

Pancreatitis. It has been shown that LPS is able to rapidly induce the expression of NUPR1a mRNA, detected by northern blot analysis in pancreatic acinar cells, in the pancreas, liver and kidneys (82). On the other hand, NUPR1 has been shown to coordinate with pancreatic associated protein-1 (PAP1) in protecting the pancreas from inflammation inducers (24). During chronic pancreatitis, NUPR1 is induced to express and protect pancreatic acinar cells from becoming apoptotic (83).

Diabetes. The loss of NUPR1 has also been observed with increases in the beta cell mass of the pancreas, suggesting that it is involved in glucose metabolism in the body (84). It has also been shown that NUPR1 plays a vital role in the protection of β -cells from apoptosis, related degradation of insulin storages and subsequent secretion during inflammatory and obesity-related tissue stress (82).

5. Therapeutic considerations

Therapeutic regulation of NUPR1. Bratland *et al* reported that vitamin 1,25(OH) $_2$ D $_3$ was able to upregulate NUPR1 expression in MCF-7 breast cancer cells and in doing so, reduce colony formation and provoke cell cycle arrest at the G1 phase, attributable to the regulation of p21Kip1 (85).

Targeting. Trifluoperazine, a drug used for psychiatric conditions has been found to interact with NUPR1 and block NUPR1-dependent tumour growth. Novel derivatives from trifluoperazine, namely ZZW-115, have been synthesised since with improved binding and potent effects on pancreatic cancer cells, providing positive prospects of novel agents in therapeutically targeting NUPR1 (7,86). Novel compounds that can target NUPR1 have been recently identified and shown to suppress pancreatic tumour development in the context of targeting NUPR1 (87). Although it is still too early to tell, it does indicate that NUPR1 would be a valuable therapeutic target in tumours closely linked to NUPR1 expression, pancreatic cancer being an excellent example. A

recent study by Deng *et al* (2017) found that a STAT3 inhibitor, fluorofenidone was able to reduce the level of NUPR1 in lung adenocarcinoma cell lines, reproducibly demonstrated *in vivo* and *in vitro*, in line with reductions in cell growth, colony formation and tumour growth (88). Specific peptides have been shown to block the interaction between NUPR1 and one of its key partner proteins RING1B, indicating a value for targeting the action of NUPR1 (89). Cationic solid lipid nanoparticles have been tested as a means with which to deliver anti-NUPR1 plasmids to HCC cancer cells and have successfully resulted in reduction of NUPR1 in these cells (90). Moreover, Lan *et al* (2020) demonstrated that ZZW-115 sensitized cancer cells to genotoxic agents by the inhibition of NUPR1 nuclear translocation, which in turn reduced the SUMOylation-dependent functions of DNA damage response proteins (6).

Chemoresistance. NUPR1 has been found to be involved in the resistance of thyroid cancer cells to Lenvatinib therapy (91). Low levels of NUPR1 in fibroblasts have been shown to result in resistance to adriamycin (79). In breast cancer cells, NUPR1 also confers resistance to chemotherapeutic agents, including Taxol and doxorubicin, by involving the NUPR1-PI3K/Akt-phospho-p21 axis (29,92). NUPR1-deficient pancreatic cancer cells are more sensitive to chemotherapeutic drugs, with the activation of NUPR1 increasing drug resistance to gemcitabine (46,93) and also becoming more sensitive to HSP90 inhibitors (94) and mTOR inhibitors in squamous cell carcinoma of skin (95). Likewise, knockdown of NUPR1 in liver cancer cells sensitises their response to sorafenib, which itself induces the expression of NUPR1, resulting in acquired resistance (12,66). However, the link may be more complex than just an NUPR1 connection. In a very interesting preliminary study, a small number of patients with cervical cancers were tested for changes in a panel of molecules involved in DNA repair and candidate drug resistance before and after two cycles of cisplatin treatment (96). NUPR1 was found to be one of the few proteins that was consistently reduced following treatment; however, this change was not connected to the clinical and pathological response. This important observation, the very first in a clinical setting, has raised a number of important questions; for example, the candidacy of NUPR1 as a drug resistance regulator in the body *in vitro* for cisplatin, leads to the question of whether NUPR1 should be considered together with other key partners. The question is whether an increase in the reduction of NUPR1 is in fact a signal for drug resistance in the context of whole body for instance. This interesting research topic, to be expanded further by the researchers, would shed important light on such questions. In a colon cancer cell line (HCT116) spheroid model, NUPR1 was found to be markedly reduced (activation z ratio-1.387) in a full nutrient environment but less reduced (z ratio-0.632) in a glucose deprived environment when cells were treated with irinotecan and chloroquine (97). Whilst this suggests that glucose is important in resistance to chemotherapy, it again raises questions as to the role of NUPR1 in the response to chemotherapy in different cancer types. In prostate cancer cell lines (DU145 and PC3) with acquired

resistance to docetaxel, single-cell RNA-sequencing determined differential clusters of sensitive vs. resistant cells (98). Protein ubiquitination was the most differentially regulated pathway and one of the top regulators was identified to be NUPR1. NUPR1 gene modifications revealed that NUPR1 conferred docetaxel resistance in both cell lines, indicating that it is a mediator of prostate cancer drug resistance and hence a target for resistance-reversal (98).

6. Challenges and future perspectives

NUPR1 is a highly interesting molecule to explore in the context of cancer, as demonstrated in the evidence gathered over the past 2 decades. It is involved in multiple aspects of cancer, from DNA repair, transcription regulation, cell cycle and death to metabolic activity of cancer cells. It also appears to be involved in a wide variety of cancer types. Clinically, there are demonstrable links between NUPR1 and disease progression and clinical outcome of patients, at least in certain cancer types. There are also early signs that it has a therapeutic value by targeting NUPR1 in the treatment of cancer. However, whilst exciting, a cautious approach is necessary until some of the key issues are resolved. The central issue is the inconsistencies in the role of NUPR1 in different cancer types and is a repeatedly occurring pattern. The following provides some perspectives for the likely reasons that this is observed.

Discrepancies in clinical and in vitro findings. Whilst NUPR1 itself has some contrasting roles in different types of cancer cells, a recently discovered family member, namely NUPR1-like or NUPR2, may provide some insight. NUPR1L closely resembles NUPR1 but has contrasting functions to NUPR1 (5,99). NUPR2 is regulated by p53 responsive elements and its expression is dependent on p53, a classic tumour suppressor (5). NUPR2 expression is also induced by p53 inducers, including serum starvation and oxaliplatin. Furthermore, NUPR1 and NUPR2 mutually suppress each other by way of the transcription of regulation. NUPR2 expression downregulates that of NUPR1 and vice versa. NUPR1 has been shown to be able to revert NUPR2 induced cell cycle arrest, making it a classic agonist-antagonist player in cells (5). NUPR2 is able to bind the same partners of NUPR1, including RING1B, at a similar affinity and most interestingly binds to NUPR1 (87). NUPR2 also appears to play an inhibitory role in colorectal cancer cells and can be targeted by miRF2277, which binds to the UTR of the human *NUPR2* gene and downregulate NUPR2, and in doing so, increase the growth and migration of cancer cells (41). Thus, the clinical association between NUPR1 and cancer would require the consideration of NUPR2, in the same setting, namely in the same cancer types and the same cohort, in order to establish a solid link.

Nuclear vs. cytoplasmic. One of the most interesting observations from published studies is the cellular location of the protein. In normal follicles of the thyroid gland, NUPR1 exclusively displays nuclear staining. The same nuclear staining is seen in follicular tumours. However, papillary tumours largely exhibit cytoplasmic staining, particularly in large tumours and tumours with lymph node metastasis (47). In breast cancer, both cytoplasmic and nuclear staining was

observed (31). These preliminary observations indicate that cytoplasmic distribution of NUPR1 in cancer cells may aid the aggressiveness and poor differentiation phenomenon of cancer cells. Pancreatic tumours also exhibit nuclear and cytoplasmic staining (42,43). Again, heavy cytoplasmic staining was seen in NSCLC cancers (17). A study with a larger cohort enabling us to comprehensively evaluate the cellular location of NUPR1 would answer these questions. In addition, further cell-based investigations to discern the functional discrepancies for cytoplasmic NUPR1 and nuclear NUPR1 was thought to be necessary. The nuclear vs. cytoplasmic localisation of NUPR1 remained an enigma until a recent study by Lan *et al* (2020), whose elegant study on the effect of ZZW-115-dependent inhibition of NUPR1 nuclear translocation by changes in SUMOylation-dependent functions of DNA damage response proteins (6).

A further point is that one should analyse NUPR1 together with its positive and negative regulators in the same study. For example, it would be invaluable to co-investigate NUPR1 and its potential partners such as ERBB2 in breast cancer (33). Finally, when considering targeting NUPR1 as a therapy, it is important to focus on the tumour type(s) that has/have a demonstrable connection, such as pancreatic cancer, whereas other cancer types would require additional work for the relationship to be firmly established. This is an exciting avenue of research that we anticipate could reveal new targets in cancer treatment in the future.

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WGJ and TAM conceived the design of the study. AXL, AJS, RH, LY and KF all contributed to the content (literature search and writing of the text) of the present review. All authors read and approved of the final manuscript.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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