Protein Quality Control and Cancerogenesis

Kontrola kvality proteinů a kancerogeneze

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Summary

Both nascent and mature proteins are prone to damaging changes induced by either external or internal stimuli. Dysfunctional or misfolded proteins cause direct physiological risk in crowded cellular environment and must be readily and efficiently eliminated. To ensure protein homeostasis, eukaryotic cells have evolved several protein quality control machineries. Protein quality control plays a special role in cancer cells. Genetic instability causing increased production of damaged and/or deregulated proteins is a hallmark of cancer cells. Therefore, intrinsic genetic instability together with hostile tumour microenvironment represents a demanding task for protein quality control machineries in tumours. Regulation of general protein turnover as well as degradation of tumour-promoting/suppressing proteins by protein quality control machineries thus represent an important processes involved in cancer development and progression. The review focuses on the description of three major protein quality control pathways and their roles in cancer.

Key words

protein quality control (PQC) – ubiquitination – endoplasmic reticulum – autophagy

Souhrn

V průběhu své syntézy i po jejím dokončení jsou buněčné proteiny vystavovány vnějším i vnitřním faktorům způsobujícím jejich poškození. Nefunkční či nesprávně složené proteiny představují přímé fyziologické riziko pro vysoce komplexní buněčné prostředí a musejí být efektivně odstraňovány. U eukaryotních buněk se vyvinulo několik mechanizmů kontroly proteinové kvality zajišťujících proteinovou homeostázu. Významnou roli hrají tyto mechanizmy v nádorových buňkách, u nichž genetická nestabilita spolu s nepříznivým prostředím nádorové tkáně vede ke zvýšené produkci poškozených nebo deregulovaných proteinů. Kontrola kvality proteinů zahrnující rovněž degradaci nádorových supresorů a onkoproteinů tak představuje důležitý proces provázející vznik a vývoj nádoru. V tomto souhrnném článku se zaměřujeme na popis tří hlavních buněčných mechanizmů kontroly kvality proteinů se zvláštním ohledem na jejich úlohu v kancerogenezi.

Klíčová slova

kontrola kvality proteinů – ubikvitinace – endoplasmatické retikulum – autofagie

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Ubiquitin-Proteasome System

The main cellular pathway involved in targeted degradation of both normal and misfolded cytosolic proteins is the ubiquitin-proteasome system (UPS), where substrates are marked by conjugation with ubiquitin protein (76 amino acids) and subsequently degraded by the proteasome, a multi-subunit protease that is localised in the nucleus and cytosol of cells. The proteasome contains one large barrel-like subunit that serves as the catalytic core and two regulatory subunits, which act as a recognition and entry site for proteins destined for proteolysis [1,2]. Protein ubiquitination is an ATP-dependent and highly organised multistep enzymatic process that requires the sequential action of three enzymes. The E1 activating enzyme uses cysteine at its active site to form a thioester bond with the C-terminal glycine of ubiquitin. Ubiquitin is then transferred from E1 to the active site cysteine of an E2 conjugating enzyme. The third step is mediated by E3 ubiquitin ligase which facilitates the transfer of ubiquitin to the protein substrate. E3 thus catalyses polyubiquitination of protein substrates and directs the protein for rapid degradation in the proteasome [3]. The E3 ubiquitin ligases, unlike E1 and E2, are specific to the protein substrate. In accordance, more than 1,000 proteins have been identified bearing E3 signatures.

The dysregulation of E3 ubiquitin ligases is often linked with human diseases, particularly cancer [4]. E3 ubiquitin ligases can trigger degradation of either oncoproteins or tumour suppressor proteins, thus they may act as either tumour suppressors or oncoproteins. As an example, role of three cancer-related E3 ubiquitin ligases MDM2, VHL and BRCA1 is described below.

p53 is an exhaustively studied tumour suppressor protein whose activity is mainly regulated by ubiquitination [5]. The principal function of p53 is to maintain genome integrity and prevent malignant transformation by transactivation of genes responsible for cell cycle arrest or apoptosis. MDM2 was discovered as the principal physiologic E3 ubiquitin ligase of mammalian p53 [6]. MDM2 is an oncogenic E3 ubiquitin ligase which binds to p53 and facilitates its ubiquitination and degradation, keeping p53 activity low in normal non-stressed cells. The signalling pathways triggered by genotoxic or other stress disrupt the interaction between MDM2 and p53 which results in p53-controlled cell cycle arrest or apoptosis [5]. The ability of p53 to prevent tumourigenesis is the reason why p53 function is restricted in most (or perhaps all) advanced cancers. MDM2 gene amplification and protein overexpression are present in more than one third of human sarcomas, breast cancer, lung cancers and other tumour types [7]. A general strategy for targeting p53 degradation induced by MDM2 is to interfere with their interaction and detach MDM2 from p53 [8]. Nutlin-3a, a small chemical inhibitor that disrupts p53-MDM2 binding, can induce cell cycle arrest or apoptosis in tumour cells expressing wild type p53 [9,10]. Other therapeutic compounds targeting p53-MDM2 interaction, RITA and MI-63, are currently in pre-clinical trials [11,12].

Another important E3 ubiquitin ligase implicated in tumour progression is VHL (von Hippel-Lindau) ligase [13]. The best known substrate of the VHL ligase is HIF-1α (Hypoxia-Inducible Factor-1α), a key mediator of oxygen homeostasis and regulator of genes in energy metabolism and angiogenesis. Under normoxic conditions, HIF-1α is permanently targeted by VLH for degradation. Under hypoxic conditions, HIF-1α escapes from VHL-induced degradation and induces the VEGF (Vascular Endothelial Growth Factor) gene, which promotes angiogenesis [14]. Mutation in VHL prevents degradation of HIF-1α under normal oxygen conditions, leading to the up-regulation of HIF-1α-induced genes which are responsible for enhanced angiogenesis in tumours [15]. The restoration of VHL ligase function would be a promising strategy to treat VHL-associated tumours.

Ubiquitination is also involved in signalling pathways triggering the DNA damage response [16]. Activity of BRCA1 E3 ubiquitin ligase is implicated in this process by ubiquitinating CtIP [16]. This ubiquitination does not lead to degradation of CtIP, but instead ubiquitinated CtIP binds to chromatin following DNA damage and is likely to be involved in DNA damage checkpoint control. Generally, BRCA1 exhibits tumour suppressor activity as its mutation is detected in more than 50% of inherited breast cancers [17]. It is clear that the E3 ubiquitin ligase activity of BRCA1 is of critical functional importance for the tumour suppressor function of BRCA1, since tumour-derived BRCA1 alleles are frequently deficient in E3 ubiquitin ligase activity [18].

Increased proteasome activity is associated with malignant disease, including those of the colon [19], prostate [20], and leukaemia [21]. Many proteasome target proteins have been identified as important mediators in tumourigenesis, including cyclins [22], tumour suppressor protein p53 [23], pRB [24] and pro-apoptotic protein Bax [20]. These facts make the proteasome a favourable target in cancer therapy. The most promising proteasome inhibitor is a dipeptide boronic acid derivative, bortezomib, which is the first therapeutic proteasome inhibitor drug tested in human patients for the treatment of relapsed and refractory multiple myeloma [25,26]. Unfortunately, bortezomib on solid tumours has not shown a satisfactory therapeutic effect to date [27]. Two other boronate-based analogues, CEP-18770 and MLN9708, are in clinical development as well as the structurally and mechanistically different inhibitor, Carfilzomib [28–30].

Endoplasmic Reticulum Protein Homeostasis and Unfolded Protein Response

Since all components of the ubiquitin-proteasome system reside in the cytosol and/or the nucleus, damaged proteins from other compartments designed for proteasomal proteolysis have to be re-translocated prior to their degradation. This process in endoplasmic reticulum is called *endoplasmic reticulum-associated degradation (ERAD)* and represents an important protein quality control (PQC) pathway as the endoplasmic reticulum produces large amounts of membrane and secretory proteins [31,32]. When the capacity of ERAD pathway is exceeded,

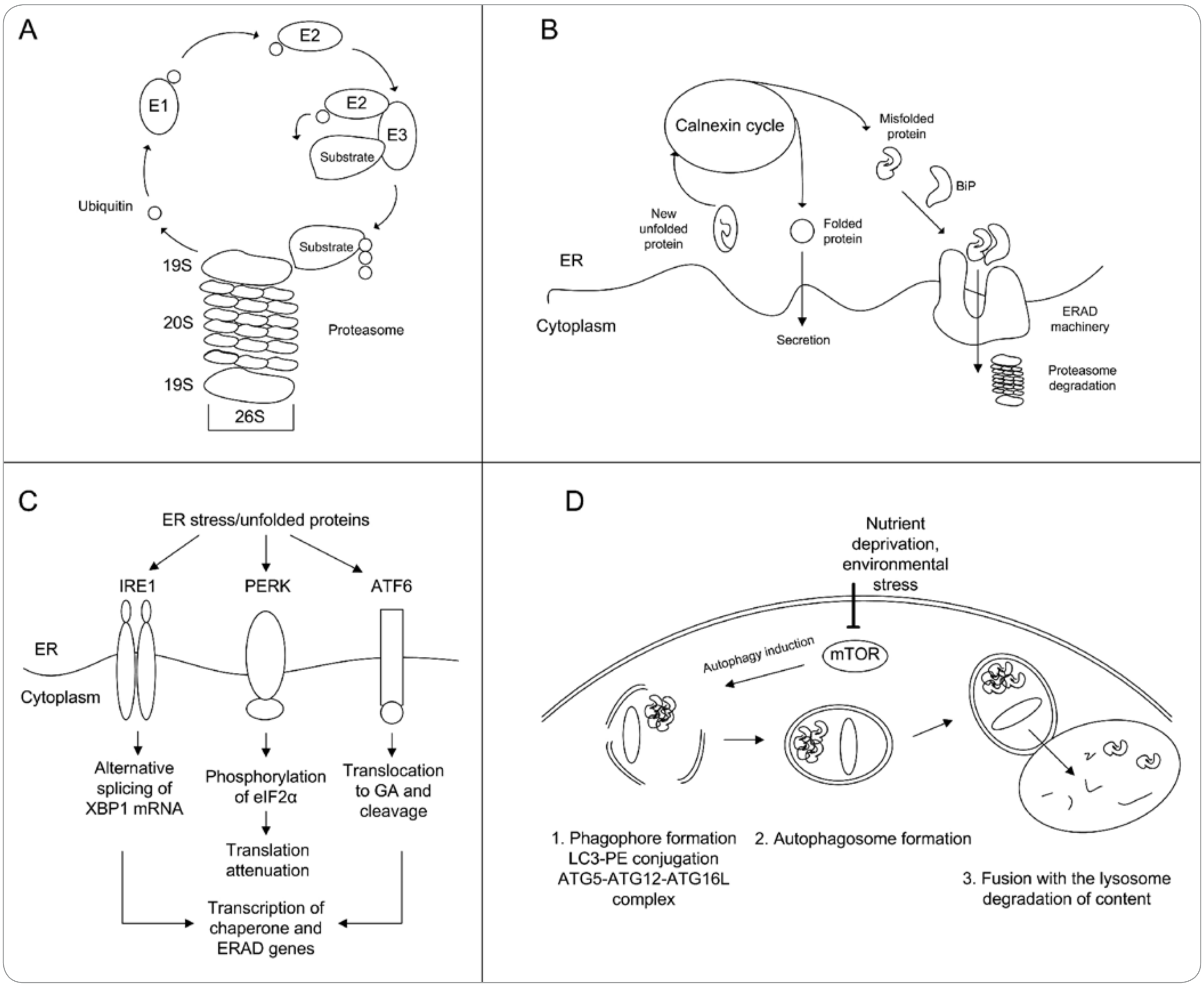

Fig. 1. Protein quality control machineries. A) Ubiquitin-proteasome pathway. Free ubiquitin molecules are activated by the E1 enzyme and transferred to E2 conjugating enzyme. An E3 ubiquitin ligase next facilitates transfer of ubiquitin to the protein substrate which is then degraded in 26S proteasome. B) Endoplasmic reticulum (ER) protein quality control. Newly synthesised proteins in the ER are glycosylated and enter the calnexin cycle to achieve correct folding. Successfully folded proteins enter the secretion pathway. Unfolded/misfolded proteins are recognised by the BiP chaperone and are subsequently ubiquitinated and transferred to the cytoplasm for proteasomal degradation by ERAD machinery proteins. C) Unfolded protein response. ER stress caused by accumulation of misfolded proteins activates membrane sensors IRE1, PERK and ATF6. Their activation triggers a cellular response leading to attenuated protein translation and increased transcription of chaperone and ERAD genes. D) Autophagy. Environmental stress and nutrient starvation inhibits activity of mTOR kinase and leads to activation of the autophagy pathway. Two protein complexes, LC3-PE and ATG5-ATG12-ATG16L, enable the formation of double-membrane phagophores and mature autophagosomes to sequester damaged organelles and aggregated proteins for degradation in lysosomes.

cells activate a survival signal response to restore endoplasmic reticulum (ER) homeostasis, known as the unfolded protein response (UPR) [33].

Most of the polypeptides synthesised in ER are glycosylated by N-linked glycans [34]. Sequential trimming of the N-glycans by ER glucosidases generates monoglucosylated glycans that are recognised by calnexin/calreticulin. These lectins protect the premature export of the nascent polypeptide chain from the ER [35]. Calnexin/calreticulin introduces the glycosylated polypeptide into a cycle where re-glucosylation of the glycans is determined by the detection of exposed hydrophobic patches [36]. After undergoing several cycles, correctly folded proteins are released from the cycle and exported from the ER. Incorrectly folded proteins are retained in the ER and processed by the ERAD pathway.

The key role in the ERAD pathway is provided by specialised E3 ubiquitin ligases targeting misfolded substrates for re-translocation and subsequent degradation in the cytosol. The first described ubiquitin ligase of ERAD was the integral transmembrane protein gp78 [37]. Knock-down of gp78 induces the accumulation of CD3 in the ER membrane, showing that gp78-mediated ubiquitination precedes re-translocation of substrates into the cytosol [38]. Sarcoma metastasis growth is inhibited by gp78 knock-down [39]. This gp78 pro-metastatic activity is probably caused by its ability to target the metastasis suppressor KAI1 for degradation. Reduced levels of gp78 increase the sensitivity of cells to cell death induced by ER stress. Suppression of KAI1 partially restores survival of gp78-deficient cells. Thus, gp78 supports metastasis by decreasing tumour cell death rate and by degrading the metastasis suppressor KAI1.

The next ER resident E3 ubiquitin ligase implicated in ERAD is Synoviolin [40]. The role of Synoviolin in cancer cells has not yet been addressed. Interestingly, gp78 is a substrate for Synoviolin ubiquitin ligase [41], supported by the observation that Synoviolin-null cells have higher steady-state level of gp78 [42]. Thus, Synoviolin may function as a metastasis suppressor by down-regulating the level of gp78. Moreover, Synoviolin has also been reported to ubiquitinate cytosolic p53 [43].

Another protein with ubiquitin ligase signatures, Trc8, was originally identified as a tumour suppressor associated with hereditary renal cell carcinoma [44]. Its overexpression in kidney cells suppresses growth *in vitro* and tumour formation in xenograft models [45]. This is due to a G2/M arrest and increased apoptosis. Overexpression of Trc8 represses genes involved in cholesterol and fatty acid biosynthesis, thus affecting the lipid synthesis necessary for rapid cancer cell proliferation [45].

Low oxygen levels, nutrient deficiency or mutations can induce accumulation of unfolded proteins in the ER and activate the UPR [33]. The UPR diminishes ER stress by induction of ERAD and ER chaperones to enhance the clearance of unfolded proteins from the ER, and by inhibition of general protein translation. Under severe ER stress, UPR can trigger apoptosis [46]. The UPR consists of 3 signalling pathways triggered from the ER. The ER stress sensors are IRE1 (inositol-requiring protein 1), PERK (PKR-like ER kinase), and ATF6 (activating transcription factor 6); all 3 are integral ER membrane proteins. These proximal sensors are activated by their dimerisation, which is prevented by binding of ER chaperone BiP (Binding immunoglobulin protein) [47]. As unfolded proteins accumulate in the ER, BiP is sequestered from these sensors, allowing their oligomerisation and activation.

IRE1 is a transmembrane protein containing an endoribonuclease domain [48]. When activated, IRE1 cleaves an alternative intron in XBP1 (X-box-binding protein 1) mRNA. This splicing leads to a frame shift and results in the translation of the spliced form of XBP1, a 41-kDa basic leucine zipper (bZIP) family transcription factor that induces genes involved in UPR and ERAD [48]. IRE1 also cleaves many mRNAs that encode secreted proteins, reducing the load of protein in the stressed ER [49]. IRE1 is also able to trigger the activation of JNK kinase [50]. The IRE1-JNK pathway is involved in ER stress-induced cell death.

PERK is a transmembrane protein in the ER with kinase activity that is triggered by oligomerisation and subsequent autophosphorylation [51]. Activated PERK phosphorylates eIF2α (eukaryotic initiating factor 2 subunit α), thus inhibiting protein translation in general. However, ATF4 mRNA translation is de-repressed when eIF2α is inhibited [52]. ATF4 promotes expression of ER chaperones and genes involved in resistance to oxidative stress [53]. On the contrary, ATF4 also induces CHOP (C/EBP homologous protein), which plays an important role in ER stress-induced cell death [54].

ATF6 is a transmembrane protein activated by regulated proteolysis. During ER stress, ATF6 is translocated to the Golgi apparatus and sequentially cleaved by the Golgi resident serine proteases [55]. This leads to release of its 50-kDa cytosolic domain functioning as a transcription factor. Upon translocation to the nucleus, the cytosolic domain induces expression of CHOP, ER chaperones and ERAD components [56].

Cancer cells in primary tumours and metastasis have to cope with inconvenient microenvironments characterised by hypoxia, nutrient deprivation and acidosis. These environmental stimuli induce ER stress which is compensated by activating the UPR.

IRE1-XBP1 axis is important for tumour cell survival and growth in hypoxic conditions as shown in xenograft models [57]. Depletion of XBP1 sensitises cells to ER stress–induced cell death and abrogates tumour growth in immunocompromised mice. Knock-down of XBP1 also reduces catalase expression and enhances ROS generation, supporting the role of IRE1-XPB1 axis in resistance to oxidative stress [58]. The levels of XBP1 activity differ between tumours, correlating inversely with their glucose availability, suggesting IRE1 activation in response to glucose starvation [59].

Another ER-stress sensor PERK is essential for tumour cell development and hypoxia tolerance [60]. PERK-deficient tumour cells show reduced viability under hypoxic conditions and form smaller tumours. PERK stabilises the transcription factor Nrf2 [61]. Nrf2, as well as ATF4, (see above) induces expression of ARE (antioxidant response elements) regulated genes, including antioxidants, cell survival and the chaperone system [62]. Thus, PERK activation enhances cancer cell defence against oxidative stress.

The above mentioned data show that the UPR enables cancer cells to adapt to increasing stress stimuli in growing primary tumours and especially during the metastatic process. Modulation of the UPR in tumours thus represents a promising therapeutic approach.

Autophagy Pathway

Autophagy means in Greek „self-eating", and refers to a cellular process engaged in lysosomal degradation of self constituents [63]. Basal autophagy helps maintain homeostasis by contributing to protein and organelle turnover, while additional autophagy is induced in stressed cells as a survival mechanism. Three types of autophagy have been described: macroautophagy [64], microautophagy [65] and chaperone-mediated autophagy (CMA) [66]. Microautophagy refers to the non-selective process whereby cytosolic proteins are sequestered by invagination of the lysosomal membrane. Chaperone-mediated autophagy is a selective process whereby proteins with defined consensus sequences are recognised by molecular chaperones, including Hsc70, and delivered to the lysosome. In this article we will focus on the role of macroautophagy.

In the process of macroautophagy (hereafter referred to as autophagy), macromolecular aggregates, portions of cytoplasm, membranes, or entire organelles are sequestered within a newly formed membrane structure, the phagophore, that subsequently forms a double-membrane vesicle (autophagosome) and fuses with lysosomes [67]. The phagophore is built by ATG (autophagy-related gene) proteins using two ubiquitin-like mechanisms [68]. First, ATG12 is conjugated to ATG5 resulting in the formation of an oligomeric ATG5-ATG12-ATG16L complex. The second reaction is the formation of the phagophore by ubiquitin-like protein LC3 (ATG8) conjugation with membrane phosphatidylethanolamine (PE). When both LC3-PE conjugates and ATG5-ATG12-ATG16L protein complex are localised to the phagophore, the formation of the autophagosome is complete [69]. Originally, autophagy was considered to be a bulk degradation pathway with no selectivity. Recent studies revealed selective degradation of organelles, proteins and protein aggregates mediated by autophagy receptors, p62 and NBR1, which are able to bind simultaneously ubiquitinated degradation cargo and LC3 [70]. The role of these autophagy receptors is particularly important during assembly of large protein aggregates, called aggresomes, that are actively formed close to microtubule organising centre (MTOC) by microtubule-dependent transport and subsequently degraded by autophagy [71].

The biochemical regulation of autophagy engages the activity of a plethora of signalling molecules [72,73]. The first signal for the formation of the autophagosome is the synthesis of phosphatidyl-inositol-3-phosphate (PI3P) molecules by the PI3K-III kinase, which becomes active upon interaction with Beclin 1 [74]. This process is negatively regulated by binding of Bcl-2 family members to Beclin1 preventing its' binding to the PI-3K-III complex and thereby reducing autophagosome formation [75]. The main inhibitor of autophagosome formation is the mammalian target of rapamycin (mTOR) pathway, a nutrient-sensing kinase pathway. Under permissive conditions the mTOR pathway is activated by PI3K-I/AKT signalling and regulates cell growth and survival. Under nutrient starvation, the mTOR pathway is inhibited by AMPK (AMP-activated protein kinase) pathway, which senses the lack of ATP, allowing induction of autophagy [76]. Mechanistically, active mTOR kinase inhibits autophagy by phosphorylating ATG1 thereby blocking autophagosome formation [77]. Experimentally, autophagy is inhibited by bafilomycinA1 or 3-methyladenine [78,79]. Bafilomycin A1 is a specific inhibitor of vacuolar-ATPase, which prevents vacuolar acidification necessary for autophagosome maturation [78] while 3-methyladenine inhibits PI3K-III kinase [79].

Targeting the autophagy pathway is in the process of evaluation as a new anti-cancer therapeutic option [80]. Data in the literature show that both autophagy enhancers and autophagy inhibitors may elicit beneficial effects by inducing cancer cell death. Autophagy may function as a tumour suppression mechanism by removing damaged compartments and proteins, thus limiting cell growth and preventing genomic instability [81]. Beclin 1 +/− mice were shown to develop malignant lesions, indicating that Beclin 1, a protein required for autophagy induction, is a haploinsufficient tumour suppressor gene [82]. Correspondingly, excessive stimulation of autophagy due to Beclin 1 overexpression can inhibit tumour development [83]. Autophagy also reduces reactive oxygen species (ROS)-triggered genomic instability by eliminating the p62 protein associated with misfolded protein aggregates and damaged mitochondria [81]. Knockdown of p62 in autophagy-defective cells prevented ROS and the DNA damage response [81].

However, more reports provide data to support the pro-tumourigenic role of autophagy [84]. siRNA-mediated depletion of ATG proteins sensitises cancer cells to radiotherapy and chemotherapy, and the autophagy inhibitors 3-methyladenine (3-MA) and bafilomycin A1 cause radiosensitisation of malignant glioma cells [84]. Furthermore, constitutive activation of the PI3K/Akt/mTOR axis that plays a decisive role in the negative regulation of autophagy, has been implicated in many human cancers [85]. The tumour suppressor protein p53 can modulate autophagy depending on its cellular localisation. Nuclear p53 acts as a transcription factor that transactivates several autophagy inducers to activate autophagy through inhibition of mTOR [86], whereas cytoplasmic p53 inhibits autophagy by activation of mTOR downstream signalling [87]. Cytoplasmic p53 also binds to high mobility group box 1 (HMGB1) preventing formation the HMGB1/Beclin 1 complex and inhibiting autophagy [88]. Moreover, p53 inhibition was found to promote cell survival in response to glucose starvation through autophagy [89]. These results suggest that the autophagy induced by p53 deletion in tumours provides a survival advantage to malignant cells in response to unfavourable conditions. More generally, it is suggested that at the precancerous stage an autophagy defect would facilitate genomic instability and tumour development, however in growing tumours the up-regulation of autophagy compensates for the limited nutrient supply and helps to combat genotoxic and metabolic stresses [90].

The ambiguous relationship between autophagy and cancer development shows the necessity to focus on regulation of autophagy at different stages of cancer and metastasis. However, it is clear that affecting autophagic protein quality control pathway is a promising approach to improve outcome of cancer treatment.

Conclusion

Folding status, abundance, localisation and activity of proteins is regulated by several mutually interconnected protein quality control machineries – ubiquitin-proteasome system, endoplasmatic reticulum-associated degradation and autophagy. Unbalanced, pathogenic function of these machineries, mostly caused by mutation, can have severe impacts on cell phenotypes and cancer is a particularly important pathology associated with malfunctioning PQC machineries. A detailed understanding of the molecular mechanisms involved in PQC may enable us to design specific pharmacological treatment of cancers with deregulated protein homeostasis. This approach has been successfully applied in for multiple myeloma by the proteasome inhibitor bortezomib and other compounds are currently being tested clinically and pre-clinically.

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