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Review

The unfolded protein response as a target for cancer therapy

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ABSTRACT

Various physiological and pathological conditions generate an accumulation of misfolded proteins in the endoplasmic reticulum (ER). This results in ER stress followed by a cellular response to cope with this stress and restore homeostasis: the unfolded protein response (UPR). Overall, the UPR leads to general translational arrest and the induction of specific factors to ensure cell survival or to mediate cell death if the stress is too severe. In multiple cancers, components of the UPR are overexpressed, indicating increased dependence on the UPR. In addition, the UPR can confer resistance to anti-cancer treatment. Therefore, modification of the UPR should be explored for its anti-cancer properties. This review discusses factors associated with the UPR that represent potential therapeutic targets.

Contents

1. Introduction

The endoplasmic reticulum (ER) is the site within the cell where proteins, steroids, cholesterol and lipids are synthesized. The ER also functions as an intracellular reservoir for Ca^{2+}. The synthesis of proteins depends heavily on these Ca^{2+}-rich conditions, as well as on the oxidizing environment of the ER. In addition, the proper synthesis of new proteins requires assistance of numerous other factors, including ER-resident chaperones, Ca^{2+}-binding proteins and folding enzymes. PDI (protein disulphide isomerase) is an important folding enzyme that forms disulphide bonds in new proteins. Hereby, PDI is reduced and subsequently oxidized by ERO1 (thiol oxidoreductase). The ultimate electron acceptor is molecular oxygen, which generates H_2O_2 and reduced glutathione [1].

Multiple adverse conditions can induce ER stress. These include hypoxia, nutrient deprivation, acidosis and certain chemicals (see Table 1). A consequence of ER stress is the incorrect folding and improper glycosylation of newly synthesized proteins. The accumulation of such unfinished proteins (proteotoxicity) triggers the unfolded protein response (UPR) [2]. The UPR is an evolutionary conserved cytoprotective

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Table 1
ER stress inducing compounds.

List of ER stress inducing compounds		Ref.
Tunicamycin	Inhibitor of N-linked glycosylation	[23,28,67,93]
Thapsigargin	Inhibitor of Ca^{2+} ATPase (SERCA), promotes Ca^{2+} from ER	[14,16,23,93,109,110]
A23187	Ca^{2+} ionophore, promotes Ca^{2+} from ER	[67,93,109]
Dithiothreitol	Reducing agent, induces protein misfolding	[111,112]
2-Deoxyglucose	Blocks glycolysis, also disrupts N-linked glycosylation.	[67]
Brefeldin A	ER-Golgi transport inhibitor	[93,110]
Proteasome inhibitors (Bortezomib, MG132)	Inhibit proteasomal protein degradation	[22,23]

response that allows cells to adapt to ER stress. This response aims to re-establish homeostasis by arresting both the cell cycle as well as general protein translation. This blocks the synthesis of even more proteins allowing the cell to dispose of the misfolded proteins. However, despite the translational halt, the UPR induces expression of specific factors required to restore homeostasis and regulate feedback mechanisms. For example, ER chaperone proteins and folding enzymes are activated. The UPR also enhances the capacity to degrade unfolded proteins by the proteasome or by autophagy. Failure of proper protein assembly and folding activates ER-associated degradation (ERAD). ERAD is an essential part of the UPR as it stimulates the degradation and removal of unfolded proteins from the ER. Proteins are loaded into the cytosol and degraded by the proteasome. However, ER stress may cause excessive accumulation of substrates for the proteasome, which impair the function of this system [3]. If the stress turns out to be too severe, the UPR can control cell fate by inducing apoptosis (see Fig. 1A).

Especially in cancer cells, stresses that induce the UPR are prevalent: within the tumour microenvironment regions deprived of oxygen and nutrients are common [4]. This leads to increased reliance of cancer cells on the UPR for survival and makes it an excellent therapeutic target. In addition, some anti-cancer therapies applied in the clinic are known to provoke therapy-induced ER stress, which further enhances the dependence of tumour cells on the UPR. In this review we discuss the factors that are involved in the UPR and how they affect tumour cells. Finally, we will highlight the current data on interfering with the UPR for therapeutic benefit in cancer.

2. Transducers of the UPR

The UPR comprises three signalling pathways that operate in parallel. The master regulators of these arms are three resident ER trans-membrane proteins: PERK (PKR-like endoplasmic reticulum kinase), IRE1 (inositol-requiring enzyme 1) and ATF6 (activating transcription factor 6) (see Fig. 2). The cytoplasmic parts of both PERK and IRE1 have kinase activity. PERK and IRE homo-dimerize and are activated by trans-autophosphorylation. In contrast, activation of ATF6 relies on translocation to the Golgi-apparatus, where it is cleaved. Cleaved ATF6 is transported into the nucleus, where it functions as a transcription factor for several genes. Before signalling through the UPR arms can take place, GRP78 (glucose-regulated protein 78) has to dissociate from the ER trans-membrane proteins.

2.1. Activation of the UPR depends on GRP78

In unstressed conditions, the luminal domains of PERK, IRE1 and ATF6 are occupied by GRP78, also known as BiP (binding immunoglobulin protein). This sterically blocks homo-dimerization of PERK and IRE1, and prevents translocation of ATF6. During ER stress, the accumulation of unfolded proteins triggers GRP78 to release PERK, IRE1 and ATF6, resulting in their activation. GRP78 binds the unfolded proteins with higher affinity and functions as an important ER chaperone assisting with proper protein folding or degradation. Recent evidence suggests that GRP78 dissociation from IRE1 is not sufficient for IRE1-activation [5]. Mutation of the luminal domain of IRE1 in yeast, which lacks the binding site for GRP78 but is still able to homo-dimerize, does not lead to permanent activity of IRE1. These data indicate that other factors besides GRP78 dissociation are necessary for the activation of IRE1, and possibly also PERK and ATF6.

Apart from the translocation of GRP78 from PERK, IRE1 and ATF6 to unfolded proteins, ER stress also induces de novo synthesis of GRP78 to assist in protein folding in the ER. Therefore, the induction of GRP78 expression is a well-established hallmark of ER stress and UPR-activation. Nevertheless, GRP78 has other functions besides its task as a chaperone. During ER stress, Ca^{2+} can be released from the ER, increasing the intracellular Ca^{2+}-concentration and potentially triggering cell death. Within the ER, GRP78 can bind Ca^{2+} and overexpression of GRP78 reduces cell death induced by Ca^{2+}-efflux of the ER [6]. In addition, GRP78 has anti-apoptotic properties. It can block caspase-mediated cell death [7] and inhibition of GRP78 expression increases apoptosis [8]. Furthermore, GRP78 inhibition sensitizes cells to hypoxia, oxidative stress and ionophore treatment [9].

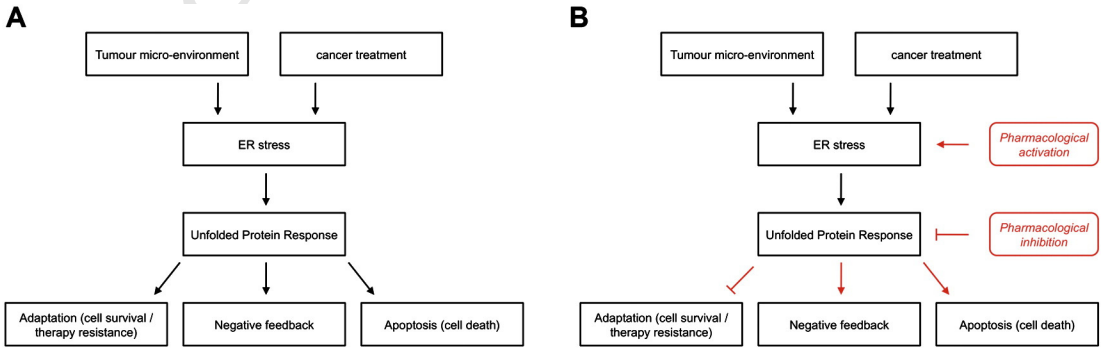

Fig. 1. The UPR induces an adaptive response after induction of ER stress, which can be therapeutically exploited. A. The UPR as a mechanism to deal with ER stress. The tumour microenvironment, as well as several cancer therapies, induces ER stress. In turn, this activates the UPR. The UPR leads to either a cellular response to cope with the stress or to cell death if the stress turns out to be too severe. In addition, a negative feedback mechanism is launched to end the response if homeostasis is restored. B. Modulation of the UPR for therapeutic benefit. Several approaches can be employed to interfere with the UPR. Firstly, ER stress can be pharmacologically activated to hyper-induce the UPR. This leads to an overload of the capacity of the UPR, aimed at the induction of cell death. Second, pharmacological inhibition of the UPR itself leads to removal of the survival benefit the UPR offers during ER stress and therefore to cell death.

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