PROGRESS IN HEMATOLOGY

The regulatory signal for normal and abnormal hematopoiesis

Regulation of unfolded protein response in hematopoietic stem cells

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Abstract

Hematopoietic stem cells (HSCs) play a central role in hematopoietic regeneration, which has been demonstrated by thorough studies. In contrast, the cell cycle status and metabolic condition of HSCs define these cells as dormant. Recent studies have also revealed that protein metabolism is quite unique, as dormant HSCs have a lower protein synthesis rate and folding capacity. Under proliferative conditions, upon hematopoietic stress, HSCs need to deal with higher requirements of protein production to achieve fast and effective blood replenishment. In such cases, increased protein synthesis could exceed the capacity of precise protein quality control, leading to the accumulation of unfolded and misfolded proteins. In turn, this triggers endoplasmic reticulum (ER) stress as a part of the unfolded protein response (UPR). Since ER stress is a multi-layered, bidirectional cellular response that contains both positive (survival) and negative (death) reactions, proper management of UPR and ER stress signals is crucial for HSCs and also for maintaining the healthy hematopoietic system. In this review, we introduce the latest findings in this emerging field within hematopoiesis and HSC regulation.

Keywords Hematopoietic stem cell · Unfolded protein response · ER stress · Proteostasis · Bile acid

Introduction

Hematopoiesis is a hierarchical process in which all types of blood cells are produced from the most immature cell type, hematopoietic stem cell (HSC) [1]. HSCs give rise to a variety of progenies for an entire life span [2]; hence maintaining their overall integrity is a top priority for a healthy hematopoietic system. In the adult body, HSCs reside in a specific microenvironment in the bone marrow (BM) where they are maintained in a dormant state under steady-state conditions [3, 4]. This dormancy is essential to avoid metabolic and genotoxic stresses due to cell divisions. In addition, recent findings have uncovered that adult HSCs have a lower protein synthesis rate and protein folding capacity in comparison with other progenies, which likely reflects the cell cycle status of dormant HSCs [5]. In contrast, under proliferative situations, e.g., during fetal development, HSCs increase their protein synthesis [6]. Due to the poor protein folding capacity, HSCs tend to accumulate un-/mis-folded proteins

and subsequent induction of the unfolded protein response (UPR), mainly endoplasmic reticulum (ER) stress response [7, 8]. ER stress induces a multiple cellular response including cell cycle arrest and apoptosis induction, which therefore is considered a natural defense system that actively eliminates cells accumulating un-/mis-folded proteins that presumably result in oncogenic transformation. Thus, higher vulnerability of HSCs to UPR has been proposed as a system to maintain a healthy HSC pool by reducing oncogenic risks [6, 9, 10]. However, under proliferative conditions, HSCs need to afford the requirements of higher protein synthesis to achieve expansion of the stem cell pool. Implication of UPR and proteostasis in HSC regulation is an emerging field. Recent studies have discovered novel mechanisms in which HSCs manage unfolded protein stress and UPR. In this review, we will describe current findings on the roles of UPR in hematopoiesis and HSC regulation.

Unfolded protein stress pathway

Proteins are the actual workhorses of genes, which govern a wide range of biological actions. The quantity of proteins not only needs to be under tight regulation, but also the

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quality/status of proteins has to be properly monitored and sensed, as disruption of the system increases the risk of serious consequences, including malignant transformation [11]. During protein production, peptides are folded with support from glycosylation and molecular chaperones [12–15], such as heat shock proteins (HSP), protein disulfide isomerases (PDI) and prefoldin complexes [12, 15, 16]. Upon stressed conditions including oxidative stress, hypoxia, starvation, inflammation, as well as overcapacity of the ER, the folding machinery can be impaired leading to the production of un-folded and mis-folded proteins [12, 13]. These abnormal proteins are labeled by glycosylation and removed through degradation pathways, including ER-associated degradation (ERAD), the ubiquitin–proteasome system, and autophagy [17, 18]. However, exceeding the capacity of protein folding and degradation results in an accumulation of un-/mis-folded proteins. Sensing such abnormal proteins is mainly governed by one of the HSP70 family members, glucose-regulated protein 78 (GRP78) [19]. Upon detection of mis-/un-folded proteins, three distinct ER stress response pathways are activated depending on the degree of the stress, triggering multiple cellular reactions (Fig. 1) [7, 8, 20]. Under relatively mild and transient stress conditions, inositol-requiring enzyme 1 (IRE1) located on the ER lumen is dimerized and phosphorylated [20, 21], which subsequently induces splicing of Xbox binding protein 1 (XBP-1) that leads to the production of the activated form (spliced XBP-1: XBP-1s) [20, 22, 23]. In addition, activating transcription factor 6 (ATF6) is translocated to golgi apparatus, and then cleaved to become an activated form (cATF6) [20, 23]. XBP-1s and cATF6 mainly govern survival signals by enhancing protein folding and degrading un-/mis-folded proteins. Under severe and continuous stress conditions, protein kinase R-like endoplasmic reticulum kinase (PERK) dimerizes and phosphorylates its downstream protein, eukaryotic translation-initiation factor 2 alpha (eIF2α), which leads to translational attenuation [20, 24]. Prolonged activation of the PERK-arm finally induces CCAAT-enhancer-binding protein homologous protein (CHOP), which mediates an apoptotic pathway through down-regulation of anti-apoptotic genes, e.g., Bcl-2 [20, 25, 26]. Thus, UPR is mediated by multiple signaling pathways that enable bidirectional roles. Some types of cells actually utilize the UPR pathway for their differentiation. For instance, secretory cells (e.g. plasma cells, pancreatic β cells etc.) require activation of UPR pathway to expand their ER mass and potential, which is essential to afford the
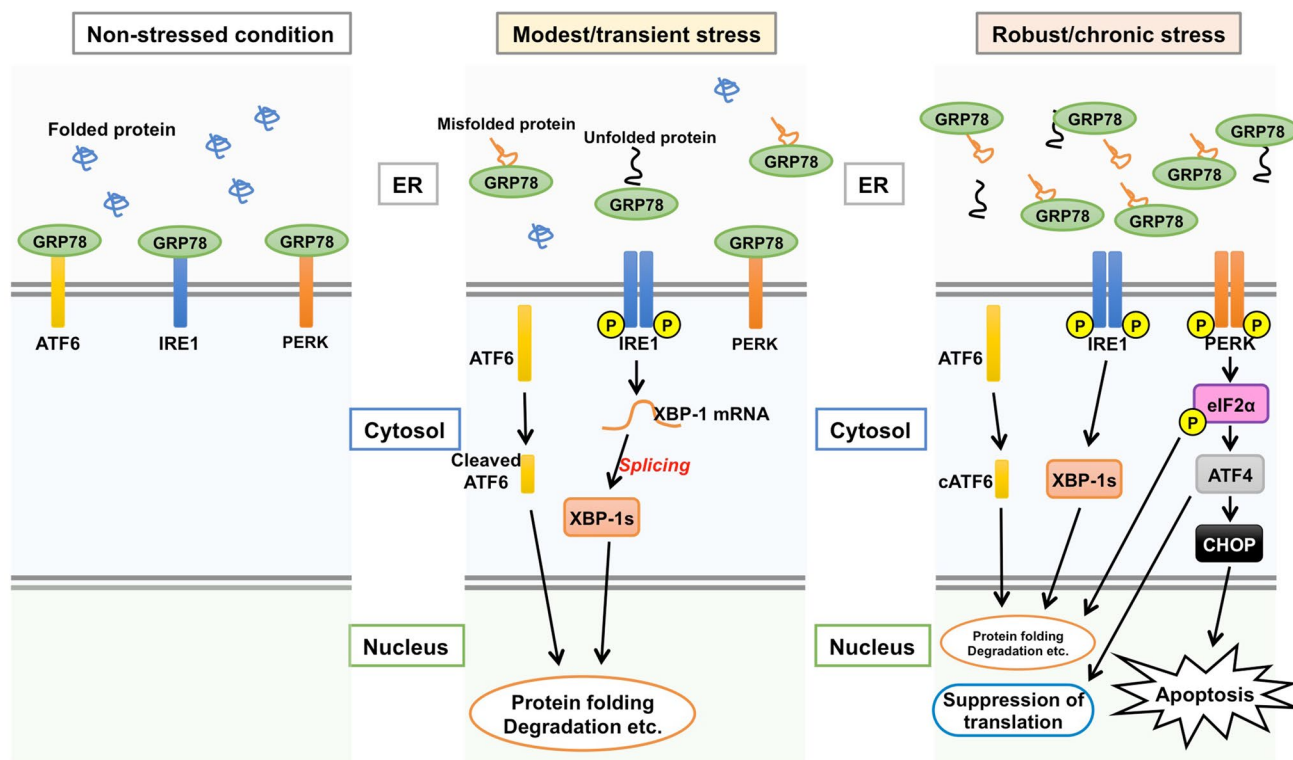

Fig. 1 ER stress pathway. Under mild and transient stress conditions, IRE1 is dimerized and phosphorylated that induces splicing of XBP-1, and ATF6 is translocated to golgi apparatus where it is cleaved to become an activated form. These proteins mainly govern survival signals by enhancing protein folding and degrading abnormal proteins. In addition, under severe and continuous stress conditions, PERK dimerizes and phosphorylates its downstream protein, eIF2α, which leads to translational attenuation and induction of CHOP that mediates an apoptotic pathway

large amount of secreted proteins being produced [27, 28]. Reimold et al. demonstrated that injection of XBP-1 knockout (KO) embryonic stem (ES) cells into Rag2 KO blastocysts restored lymphopoiesis in chimeric mice, but the B cells repopulated in the mice were unable to differentiate to plasma cells and to secrete immunoglobulin [29], indicating that ER stress pathways also serve as essential parts of cellular differentiation.

Interestingly, recent studies have discovered that ER stress pathways can be activated without un-/mis-folded proteins, but with aberrant lipid composition of the ER membrane (lipid bilayer stress: LB stress) [30, 31]. In these cases, alterations in the lipid components of the lipid bilayer, e.g., fatty acids, directly stimulates ER membrane-embedded signaling molecules and initiates the same stress pathways. Therefore, it will be important in future studies to carefully assess what is the actual cause of the stress responses.

ER stress regulation in steady-state HSCs

It remains unclear whether UPR is activated in adult HSCs. The lower protein synthesis rate is considered beneficial to avert a situation that requirement of protein production exceeds the folding capacity. On the other hand, hypoxic conditions in which HSCs are maintained [32, 33] are known to induce UPR [34]. In contrast, Rouault-Pierre et al. demonstrated that knockdown of hypoxia-inducible factor 2α (HIF2α) rather elevates ER stress-related apoptosis [35]. Analysis of ER stress reporter mice (ERAI mice) [36] and expression levels of UPR-related genes suggest late ER stress signal is not activated under steady-state conditions; however, some of the key regulators, e.g., ATF4, Gadd34 (Ppp1r15a), are highly expressed in steady-state HSCs [9, 10]. Since UPR regulation is crucial for a broad type of organs/tissues, many mouse models lacking UPR-related genes are embryonic lethal and thereby difficult to specifically study the impact on hematopoiesis. *Atf4* KO mice have been reported to contain less number of functional HSCs in the FL, although this might be a mixed phenotype from cell intrinsic and extrinsic effects, as FL stromal cells also exhibit functional defects [37]. Deletion of IRE1a, a more ubiquitous form of IRE1, also results in embryonic lethality mainly due to placental malformation [38]. *Xbp1* KO mice are also embryonic lethal due to hypoplastic fetal livers that result in severe anemia [39]. Single deletion of each ATF6 subtype, ATF6α and ATF6β, does not result in a severe phenotype while double KO mice are embryonic lethal [40]. In contrast, CHOP (*Ddit3*) KO mice exhibit minor phenotypes under steady-state conditions which could be expected considering its role in the late ER stress response [41]. These reports indicate that the early ER stress pathway is likely activated, at least during fetal hematopoiesis, but not the late/apoptotic pathway. It is of high interest how only a part of such a cascade-like signaling can be activated without triggering the next arm.

ER stress induction upon in vitro culture of HSCs

Despite the extensive in vivo potential, it remains problematic to efficiently expand HSCs in vitro [42]. One of the reasons may be the cellular status of steady-state HSCs, which are predominantly kept in a dormant state within adult BM [2, 33, 43]. Since in vitro cell culture aims to stimulate proliferation, the condition induces a variety of stress signals including oxidative stress. We discovered that adult BM HSCs have lower expression levels of chaperone proteins (e.g. HSP70, HSP60, GRP94, PDI etc.) than more differentiated progenitors thereby less capable to fold proteins, which makes HSCs predisposed to ER stress activation [9]. In fact, expression of PERK-arm genes, particularly CHOP, were preferentially induced in HSCs upon in vitro culture. We also demonstrated that overexpression of an RNA binding protein, Dppa5, can suppress the ER stress elevation in cultured HSCs, enabling maintenance of functional HSCs for 2 weeks. Conversely, knockdown of Dppa5 in HSCs using shRNA abolished long-term reconstitution capacity of HSCs, and up-regulation of ER stress pathway genes were detected without in vitro culture. These findings indicate that proper management of UPR is critical and sufficient to maintain HSCs in vitro [9]. Furthermore, we could show that the taurine-conjugated bile acid, tauroursodeoxycholic acid (TUDCA), reported as molecular chaperone [44], is potent to reduce ER stress levels in culture and therefore sufficient to support functional HSCs in vitro (Fig. 2) [9]. These findings are particularly important, as it indicates that elimination of stress accompanied with cellular proliferation is an alternative approach to support in vitro expansion of HSCs.

ER stress regulation in HSCs during development

While HSCs are maintained in a dormant state within adult BM, fetal liver (FL) is known as the organ where HSCs actively expand [45, 46]. However, it is a longtime question why HSCs can be expanded so efficiently only in FL. Consistent with the enhanced cellular proliferation, FL-HSCs showed increased protein production rate [6]. Despite this, FL-HSCs did not show any signs of elevated ER stress. This was not due to enlargement of endogenous chaperone function, as expression levels of major HSP and PDI were unchanged. Then, how do HSCs manage active expansion
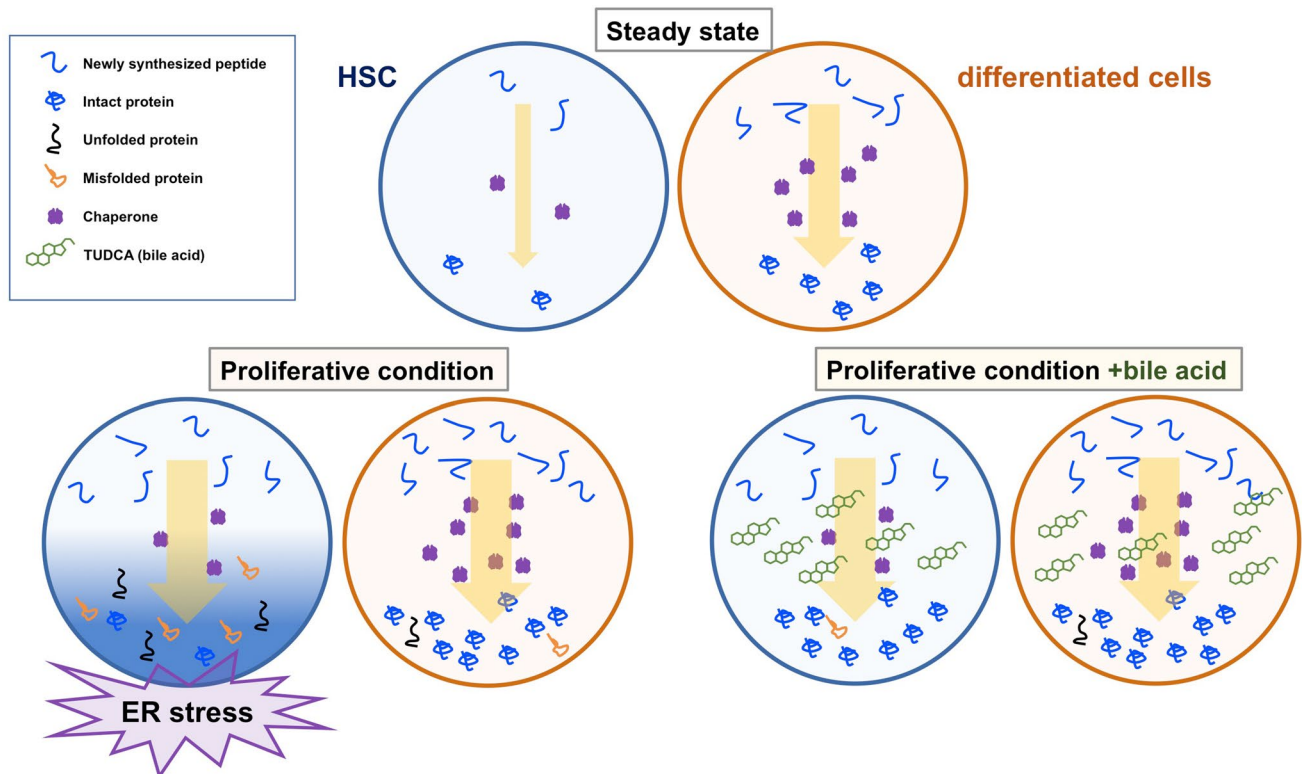
Fig. 2 Vulnerability of HSC to the accumulation of un-/mis-folded proteins. Adult HSCs under steady-state conditions have a lower protein synthesis rate and protein folding capacity in comparison with other progenies. In contrast, under proliferative situations, HSCs increase their protein synthesis. However, due to the poor protein folding capacity, HSCs tend to accumulate un-/mis-folded proteins and subsequent induction of ER stress. Supporting the protein folding capacity by adding chemical chaperines, e.g., tauroursodeoxycholic acid (TUDCA), the accumulation of abnormal proteins and the induction of ER stress is inhibited

without inducing ER stress? Surprisingly, the answer was bile acids.

Bile acid (BA) is a main component of bile, primary role of which is lipid digestion and absorption, but recently other functions as signaling molecules and chemical chaperones have been discovered. TUDCA, the chemical chaperone that we successfully used to reduce ER stress level in vitro, is also one specific type of BA. Interestingly, it has been known that FL already contains BA without having a known role since fetuses do not digest food [47, 48]. Furthermore, during the peak of fetal HSC expansion (E14.5–E15.5 in mice) [45] the bile duct structure which anatomically separates bile flow and blood flow is not structured in the liver [49], meaning that hematopoietic (stem) cells are exposed to BA in FL. We analyzed *Cyp27a1* knockout (KO) mice that lack one of the key BA synthetic enzymes [50], and interestingly KO fetuses grown in KO mothers exhibited increased ER stress levels and a significant reduction in HSC numbers [6]. The phenotype was rescued by injection of an ER stress inhibitor, Salubrinal [51], confirming the implication of the ER stress pathway. Measurement of aggregated proteins (aggresome) [52] showed that BA inhibited formation of aggresome both upon in vitro culture and in the KO mice with reduced BA

pool in FL, which may be the key mechanism of ER stress management by BA. Of note, KO fetuses derived from heterozygous mothers only showed a marginal defect, indicating that BA transported from mother is the main source of FL BA. In summary, this indicates that maternal and fetal BA coordinately suppress production of un-/mis-folded proteins in the growing FL HSCs [6].

ER stress regulation during hematopoietic regeneration

In contrast to steady-state conditions, ER stress may play a crucial role in stress hematopoiesis during the regenerative process. Our latest study has demonstrated that suppression of ER stress induction using Salubrinal significantly improved the BM recovery after chemotoxic myeloablation upon 5-fluorouracil (5-FU) injection. In addition, CHOP KO mice seem to recover better from myelosuppresive conditions. In addition, we have found that BA levels are transiently but significantly elevated in circulating blood of the mice treated with 5-FU. Analyses using *Cyp8b1* KO mice, lacking cholic acid synthesis [53], showed delayed recovery

of BM and PB from myelosuppression. In contrast, injection of TUDCA significantly improved the hematopoietic recovery from myelosuppresive conditions. Of note, TUDCA injection did not result in synergistic effect in CHOP KO mice, suggesting these two outcomes are based on the same mechanism. These findings may suggest that under stress conditions, the endogenous ER stress management system supports hematopoietic regeneration at a certain level, but the effect is limited. BA may be an extrinsic intermediary of the ER stress regulation and supplementation of more potent BA could become a supportive treatment for patients treated with chemotherapy (unpublished data).

In addition, Chapple et al. recently demonstrated that estrogen, which governs cell cycle progression of HSCs in females [54], regulates regeneration capacity of HSCs through ER stress pathway [55]. Uniquely, estrogen binds to a promoter region of IRE1α and directly controls the expression of IRE1α, but not triggering the late PERK-arm pathway. This might potentially explain how the multi-branch pathway is differentially controlled.

Considering the biological role of ER stress-related apoptotic pathway in selectively eliminating damaged cells, inhibiting the ER stress pathway might not be an appropriate approach since it has a risk to rather maintain prediseased cells. In contrast, the use of chemical chaperones, e.g., TUDCA, could be better treatments as it targets the cause of ER stress by blocking the formation of abnormal proteins (Fig. 3).

UPR in HSC ageing

Aging of HSCs is described with several representative phenotypes, including (1) increased frequency of phenotypic HSCs, (2) reduced number of functional HSCs, and skewed differentiation towards myeloid cells [56]. UPR has been proposed to be a crucial part of aging triggers/hallmarks [57] although many of the studies are not conclusive, as often only expression levels of ER stress markers are used as the main readout. For instance, our comparison of gene expression profiles using gene set enrichment analysis (GSEA) indicates that a gene signature of ER stress pathway is significantly enriched in aged HSCs compared to young HSCs; however, the enrichment is modest and altered genes are different from ones found in cultured HSCs that show much clearer induction of ER stress (unpublished data). Thus, implication of UPR is highly likely, but still not fully supported by evidence.

Recently roles of mitochondria in HSC function have been highlighted [58–61]. It has been suggested that mitochondrial proteostasis is implicated in cellular senescence and aging through mitochondrial UPR (UPR^{mt}) [62]. Mohrin et al. demonstrated that Sirt7 deletion in HSCs elevates UPR^{mt} that results in impaired regenerative potential and compromised lymphopoiesis of HSCs [63, 64]. Sirt7 expression is down-regulated upon aging, elevation of UPR^{mt} may therefore be one of the key mechanisms
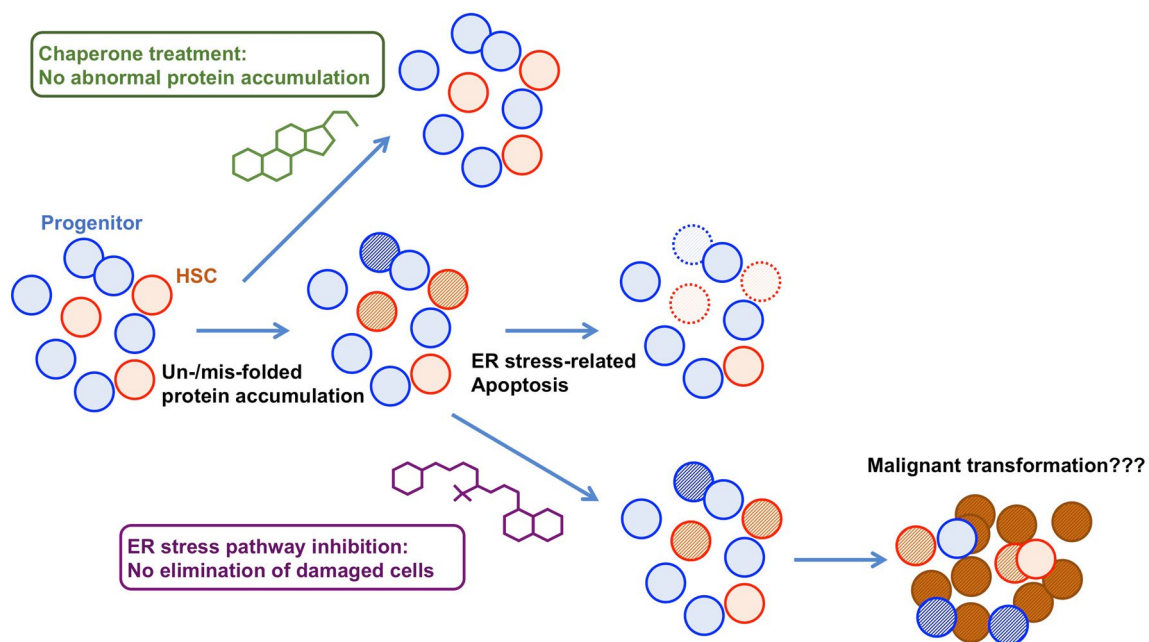

Fig. 3 Reducing the formation of un-/mis-folded proteins vs. inhibiting the apoptotic ER stress pathways. Since ER stress-related apoptotic pathway serves to remove cells accumulating abnormal proteins, inhibiting the pathway might not be an appropriate approach although it could rescue such cells from apoptosis. It therefore has a risk to maintain pre-diseased cells. In contrast, chemical chaperones alleviate ER stress through inhibiting the formation of mis-/un-folded proteins, hence the "cause" of the stress can be removed

of HSC aging. Importantly, mitochondria play a crucial role in metabolic regulation, therefore connecting UPR and metabolic alterations, as well as the oxidative stress pathway.

Concluding remarks

Although UPR has been studied well in other fields of medical and cellular sciences, it is still an emerging field in hematopoiesis and stem cell biology. Current discoveries of novel intrinsic/extrinsic regulators of UPR have the potential to provide new aspects of understanding for HSC regulation and pathogenic hematopoiesis. In addition, UPR management may be one of the key mechanisms enabling in vitro HSC expansion and modification.

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