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SURVEY AND SUMMARY

Translational alterations in pancreatic cancer: a central role for the integrated stress response

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ABSTRACT

mRNA translation is a key mechanism for cancer cell proliferation and stress adaptation. Regulation of this machinery implicates upstream pathways such as PI3K/AKT/mTOR, RAS/MEK/ERK and the integrated stress response (ISR), principally coordinating the translation initiation step. During the last decade, dysregulation of the mRNA translation process in pancreatic cancer has been widely reported, and shown to critically impact on cancer initiation, development and survival. This includes translation dysregulation of mRNAs encoding oncogenes and tumor suppressors. Hence, cancer cells survive a stressful microenvironment through a flexible regulation of translation initiation for rapid adaptation. The ISR pathway has an important role in chemoresistance and shows high potential therapeutic interest. Despite the numerous translational alterations reported in pancreatic cancer, their consequences are greatly underestimated. In this review, we summarize the different translation dysregulations described in pancreatic cancer, which make it invulnerable, as well as the latest drug discoveries bringing a glimmer of hope.

INTRODUCTION

Among all organs, the pancreas has the biggest protein synthesis capacity (1). Translation is the most energy-consuming process in cells (2) and thus requires active control to maintain energetic balance. As translational control occurs mostly at the level of the initiation step, molecular details of this stage have been extensively studied in general (3) and in the pancreas (reviewed in 4). Similarly, in pancreatic cancer (e.g. pancreatic ductal adenocarcinoma, hereafter referred as PDA), numerous findings over the past two decades have illustrated that translational control can favor cancer initiation, development, resistance to hypoxia, nutrient starvation and chemotherapies. Dysregulation of protein synthesis is considered as a hallmark of cancer cells, together with proliferation, survival and metastatic progression (5). This review synthesizes current knowledge on important translation dysregulations in PDA, mainly focusing on the initiation step, and highlights potential underlying therapeutic vulnerabilities.

PANCREATIC DUCTAL ADENOCARCINOMA

With an overall 5-year survival rate not exceeding 9%, PDA represents the seventh most common cause of cancer-related death in the world, and is predicted to become the second most common cause of cancer-related death by 2040 in the USA (6). Tobacco, alcohol, family history and genetic factors are known risk factors (7) and, more importantly, new-onset diabetes mellitus and obesity have been associated with PDA (8). PDA initiation and development is driven by KRAS oncogenic mutation (present in >90% of cases) and by functional alterations of tumor suppressor genes p16INK4A, p53 and SMAD4 (9). To date, surgery remains the only potential cure for this cancer; however, 80% of PDA tumors are unresectable, as patients are diagnosed at an advanced stage, due to a late diagnosis. First-line treatments for PDA patients include FOLFIRINOX (combination of 5FU, Leucovirin, Oxaliplatin and Irinotecan), Gemcitabine alone or in combination with nab-Paclitaxel, which are selected solely based on the patient's physical condition. Nonetheless, patient survival remains very low: respectively 11.1 versus 6.8 versus 8.5 months, showing the urgency in discovering new therapeutic strategies (7). PDA initiation and development have been explored in genetically engineered murine models (GEMMs). These models, express-

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ing mutated *KRAS* specifically in the pancreas (hereafter referred to as KC), recapitulate key features of the human disease, including histological architecture and chemoresistance. Association of mutated *TP53* with *KRAS* accelerates PDA development kinetics in GEMMs (hereafter referred to as KPC) (10).

THE TRANSLATION INITIATION MACHINERY

Most nuclear-encoded mRNAs are translated via a cap-dependent process in eukaryotic cells. The process starts by the recognition of the 5′ cap structure (7-methyl-GTP) of the mRNA by the eukaryotic translation initiation factor (eIF) 4F complex, which facilitates subsequent recruitment of the small ribosomal subunit 40S to the mRNA (3). eIF4F is composed of three proteins: eIF4E, the cap-binding subunit; eIF4G, a scaffolding protein; and the ATP-dependent RNA helicase eIF4A (see Figure 1). eIF4E is considered as the least abundant component and therefore the limiting factor for eIF4F complex assembly. eIF4E, eIF4G and eIF4A abundance controls translation of a distinct subset of mRNAs (including many oncogenes and cell cycle regulators) rather than global protein synthesis *per se*. eIF4A constitutes the only enzyme of eIF4F which unwinds RNA secondary structures through an ATP-dependent mechanism (3). The 40S ribosomal subunit is associated with the eIF3 complex (composed of 13 subunits: 3a to 3j) (11), eIF1, eIF1A, eIF5 and the ternary complex (TC) comprising eIF2 with an initiator methionyl-tRNA (Met-tRNA$_i$) to form the 43S pre-initiation complex. The 43S complex can then be tethered to the mRNA, through eIF4F, to form the 48S complex (see Figure 1), which will finally allow scanning to occur. Importantly, eIF5A1/2, also involved in translation elongation and mRNA transport, are regulated by hypusine modification, a specific and rare post-translational modification. Once the ribosome recognizes the start codon, initiation factors are released for recycling, allowing the recruitment of the 60S ribosomal subunit to form the 80S complex.

DYSREGULATION OF THE TRANSLATION INITIATION MECHANISM IN PDA

Up-regulation of translation initiation factors is a common alteration in cancers (5,12). Immunohistochemistry revealed that 85% of PDA samples have high expression of eIF4E without being correlated with either Tumor/Node/Metastasis stage (T/N/M) or overall survival (13). However, another study suggests that eIF4E expression is increased in poorly differentiated PDA and in metastasis (14). Similarly, eIF4G1 mRNA was found to be up-regulated in PDA as compared with healthy tissue and was associated with poor survival in the TCGA cohort and GEO database (15,16). High eIF4A1 protein abundance was also correlated with poor patient survival and a mesenchymal phenotype (17). In addition, eIF4E and eIF4A have been demonstrated to play critical roles in PDA metabolism. Upon KRAS stimulation, eIF4A and eIF4E were shown to activate the translation of mRNAs encoding the small GTPase ARF6 and its downstream effector AMAP1, thus promoting tumor invasion and metastasis (18). Expression and activity of initiation factors are regulated through multiple mechanisms. For example, Myc is activated or amplified in a high proportion of PDA patients (19) and can favor expression of eIF4E mRNA in a feed-forward manner (20), which suggests a potential enhancement of protein synthesis in this context. Curiously, phosphoglycerate dehydrogenase (PHGDH), the first enzyme in the serine *de novo* biosynthesis pathway, was shown to favor mRNA translation through a direct interaction with eIF4E and eIF4A, and to enhance eIF4F complex formation. Moreover, depleting or inhibiting PHGDH led to a reduced cap-bound eIF4F and a global decrease in protein synthesis (21). This last example highlights the close connection between mRNA translation and metabolism. Overall, these findings indicate a general overexpression of eIF4F components in PDA, similar to other cancers.

Alterations in the expression of specific eIF3 subunits are also found during PDA development. The eIF3a subunit is up-regulated in PDA whereas eIF3f is down-regulated compared with normal tissues (22–24). Silencing eIF3a, 3b or 3c markedly reduced pancreatic cancer cell proliferation and motility, favoring apoptosis (24–26). In contrast to the core subunit of eIF3, eIF3f silencing was shown to enhance translation and to reduce staurosporine-induced apoptosis (27). Recently, the eIF3 complex was implicated in a special mode of translation of WT1 Associated Protein (WATP) mRNA, involving m6-A modification, and leading to an enhanced WT signaling and tumor growth (28). Finally, the essential role of eIF3 in mRNA translation in PDA was reinforced in KPC GEMMs deleted for the master redox transcription factor, NRF2. KPC mice lacking NRF2 showed enhanced reactive oxygen species (ROS) production and oxidation of the translation machinery (including eIF3), associated with impaired protein synthesis. The lack of NRF2 is also associated with a reduced autocrine EGFR/Akt/4E-BP1 signaling, further decreasing the assembly of the translation initiation complex. Overall, these data indicate that protein synthesis sustains PDA growth (29).

Another factor, eIF5A, was found to be up-regulated and activated (by hypusination) in human PDA and KC GEMMs through a mechanism involving RAS mutation (30). The importance of eIF5A expression for PDA cancer cell growth was demonstrated *in vitro* and in orthotopic tumors. In addition, inhibitors of hypusination also suppressed PDA cell growth (31). Aside from a recent immunohistochemistry-based study, which identified down-regulation of eIF1, eIF2α and eIF6 to be associated with good prognosis (23), other initiation factors of the 48S pre-initiation complex have not been explored in PDA. All these examples illustrate how modification of translation initiation factors can modulate pancreatic cancer cell capacities and reinforce interest in targeting the protein synthesis machinery.

Targeting the translation mechanism in PDA

Considered as the first step of protein synthesis, targeting the assembly and/or the activity of the eIF4F complex has always been an exciting challenge, which began by limiting the activity of eIF4E, the cap-binding protein. Antisense oligonucleotide (ASO) targeting eIF4E induced the reduction of eIF4E by affecting both mRNA stability and translation. Originally, OGX-427, an ASO

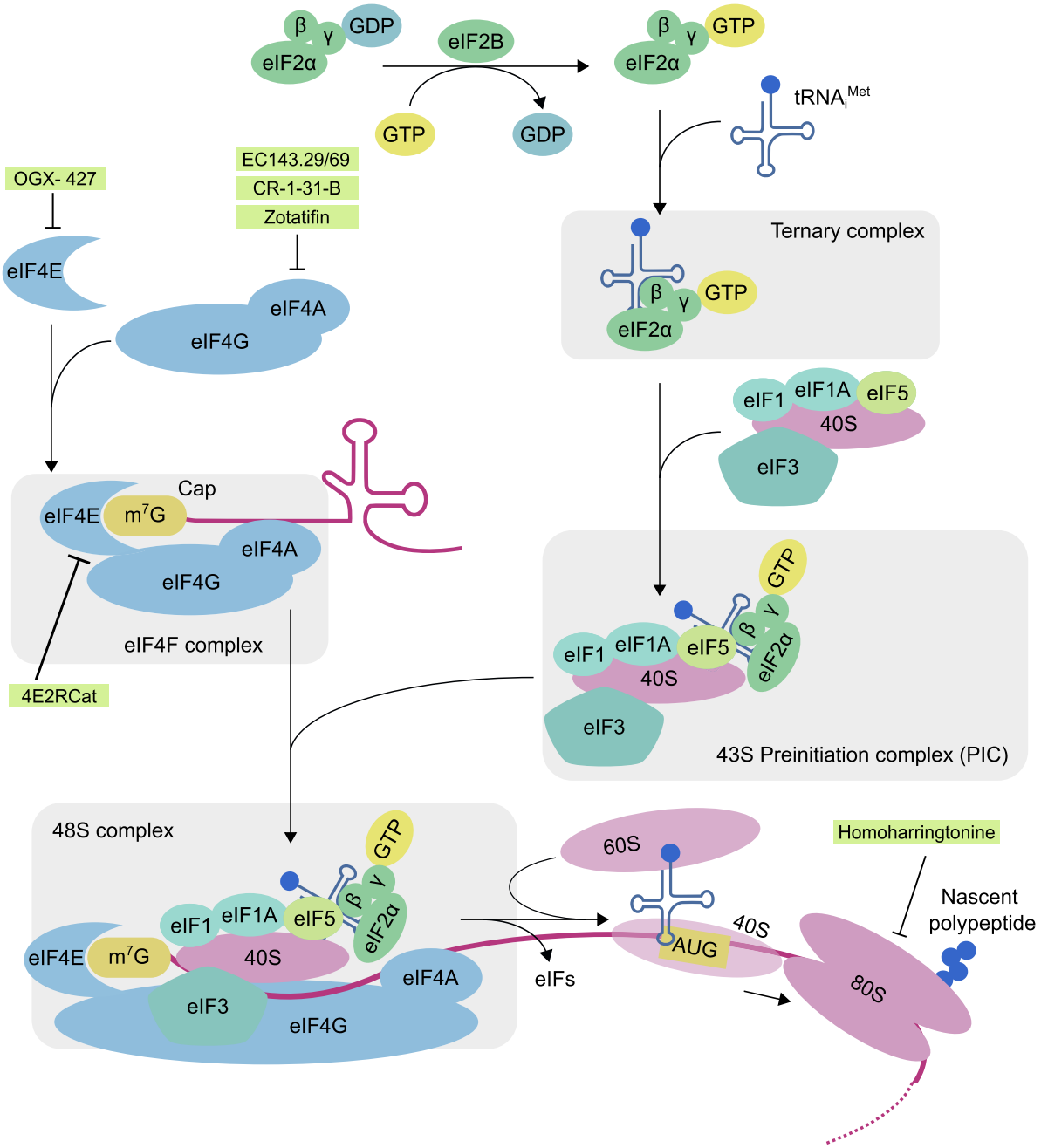

Figure 1. Overview of the translation initiation mechanism. The 5′ cap-dependent translation implies recognition of the START codon by a ribosome carrying the initiator methionyl-tRNA (Met-tRNA$_i$). First, the ternary complex (TC) is formed by the association between Met-tRNA$_i$ and eIF2. The main role of the TC is the transfer of Met-tRNA$_i$ to the 40S ribosomal subunit associated with numerous initiation factors (eIF1, eIF1A, eIF5 and eIF3). The 43 pre-initiation complex (43S PIC) is formed by the association of the TC with the 40S ribosome, through the interaction between eIF2β and eIF5. The eIF4F complex, composed of the cap-binding protein eIF4E, eIF4G and the RNA helicase eIF4A, is assembled at the cap structure. The eIF4F complex recruits the 43S PIC through the interaction between eIF3 and eIF4G to constitute the 48S complex. This complex scans the mRNA from 5′ to 3′ until the START codon, then eIF5 hydrolyzes GTP-bound eIF2γ to dissociate the 48S complex and allow association of the 60S with the 40S in order to form the 80S ribosomal subunit. Therapeutic agents under ongoing investigation, targeting this machinery, are noted in green boxes.

leading to eIF4E down-regulation, demonstrated the ability to enhance Gemcitabine activity both *in vitro* and in xenograft models (14). Unfortunately, a subsequent clinical trial with OGX-427 in combination with Gemcitabine and nab-Paclitaxel failed to demonstrate benefits for the patients (NCT01844817). An alternative to eIF4E silencing is to limit eIF4F formation at the cap structure. Using 4E2RCat, a small molecule which blocks eIF4E–eIF4G interaction, our work demonstrated that the growth of mTOR inhibitor-resistant PDA cancer cells can be reduced (32). Furthermore, this molecule was also well tolerated *in vivo* and was showed to sensitize tumors to chemotherapies in other cancers (33).

Targeting eIF4A activity has recently demonstrated an impressive efficacy in blocking protein synthesis and PDA tumor growth. Translatome analysis of KPC-derived organoids treated with CR-1-31-B, a synthetic rocaglate, revealed an oncogenic translation program supported by eIF4A, favoring expression of enzymes from glutathione metabolism, glucose uptake, oxidative phosphorylation and glycolysis (34). As a result, treatment with CR-1-31-B suppressed tumor growth and extended survival of KPC GEMMs (34), and, more recently, blocked PDA tumor progression in orthotopic and metastatic models (35). In both cases, c-MYC expression was strongly reduced following eIF4A inhibition (17,35). A reduced translation rate of KRAS and c-Myc mRNA via CR-1-31-B could be mediated by the presence of G-quadruplex structures (35). Nonetheless, many reports have pointed out that eIF4A-sensitive transcripts have larger RNA structures due to an increased 5′-untranslated region (UTR) length (36). Similarly, our data indicated that other rocaglate derivatives, EC143.29 and EC143.69, effectively reduce mRNA translation of CDC6, a core component of pre-replicative complexes, leading to DNA replication arrest *in vitro*, and blocking tumor growth *in vivo* (37). Supporting these encouraging results, a clinical trial using Zotatifin (*eFT226*), an inhibitor of eIF4A, is ongoing on solid tumors, including PDA (NCT04092673). Altogether, translation initiation factors have been widely studied, and have been shown to be globally overexpressed in PDA, contributing to cancer development, metastasis and survival. Reducing the expression of the limiting factor eIF4E has failed, but has to be carefully considered, as ASO technology is probably not the most potent approach as opposed to small molecules acting on enzymatic activity or protein interaction. The expression of eIF4E partners and regulators, such as MNK or 4E-BPs, should also be taken into account (see next paragraphs). Conversely, inhibiting the formation of the eIF4F complex through the helicase eIF4A seems to have promising results. Correlations between IHC-analyzed expression of initiation factors and patient survival remain to be functionally and molecularly deciphered in order to reveal their therapeutic potential.

Oncogenic signaling pathways regulating translation in PDA

Oncogenic mutation of KRAS is present in >90% of PDA, and thus it is considered as the driver mutation of this cancer (9). Mutated KRAS can activate two pathways: the mitogen-activated protein kinase (MAPK) pathway including MEK/ERK and p38/MAPK; and the PI3K/AKT/mTOR pathway (see Figure 2). In other cancers, activation of these pathways has been largely described to induce dysregulation of protein synthesis through translation of a subset of mRNAs encoding tumor-promoting and survival factors (5,12).

Surprisingly, few publications have looked in detail into the global protein synthesis inhibition or specific modifications of mRNA translation upon modulation of mTOR and MAPK pathways in PDA.

mTORC1 phosphorylates many fundamental factors involved in translational control, including p70-S6 Kinase 1 and 2 (p70S6K1/2), and eIF4E-binding proteins (4E-BPs) (see Figure 2), which have been poorly explored in PDA. Nearly 75% of PDA presents activation of the mTORC1 pathway as evidenced by immunohistochemical analysis of p70S6K or RPS6 phosphorylation (38). This activation of mTOR seems critical for PDA development, as evidenced by slow tumor progression in KC GEMMs harboring non-phosphorylatable sites of RPS6 (39). On the other hand, 4E-BPs (4E-BP1, 2 and 3), more specifically 4E-BP1, the best-characterized and prototypical factor, are inhibitors of eIF4F complex formation and thus of translation initiation. Upon mTORC1 inhibition, these factors are dephosphorylated and therefore sequester eIF4E away from eIF4G, thus inducing mRNA translation inhibition (40) (see Figure 2). Like p70S6Ks, 4E-BP1 phosphorylation is often monitored as a proxy of mTORC1 activity and serves to estimate the cap-dependent translation inhibition. Interestingly, our data indicate a loss of 4E-BP1 expression in PDA cancer cells from KC GEMMs as well as in 50% of human PDA samples (32). So far, this observation has only been described for PDA and head and neck squamous cell carcinomas (41). 4E-BP1 loss takes place during early PDA development and favors proliferation through uncontrolled translation of cyclin D1 mRNA, which is insensitive to mTOR inhibitors (32). A broader analysis of the impact of 4E-BP1 loss on genome-wide mRNA translation revealed enhanced DNA replication and repair processes, mediated by CDC6 and RRM2, two components of the replication machinery. Mechanistically, CDC6 and RRM2 mRNA translation became uncontrolled upon 4E-BP1 down-regulation, as well as insensitive to mTOR inhibitors (37). Other reports have correlated 4E-BP1 expression and dephosphorylation to the efficacy of TRAIL-induced apoptosis, alone or in combination with Gemcitabine (42,43). These effects were associated with the decrease of global protein synthesis rate rather than with translation inhibition of specific mRNA targets.

KRAS leads to the activation of MNK1/2, downstream of MEK/ERK and p38/MAPK, which phosphorylates eIF4E, allowing translation of a subset of mRNAs implicated in tumor development, epithelial to mesenchymal transition and migration (see Figure 2) (44,45). In the absence of MNK1 (and consecutive absence of eIF4E phosphorylation), mouse pancreata display normal histology, despite an impaired homeostatic response to acute pancreatitis (46), one major risk factor for PDA development (7). Curiously, mice carrying non-phosphorylatable eIF4E alleles (eIF4ES209A/S209A) showed normal response to experimental pancreatitis (generated by serial caerulein in-

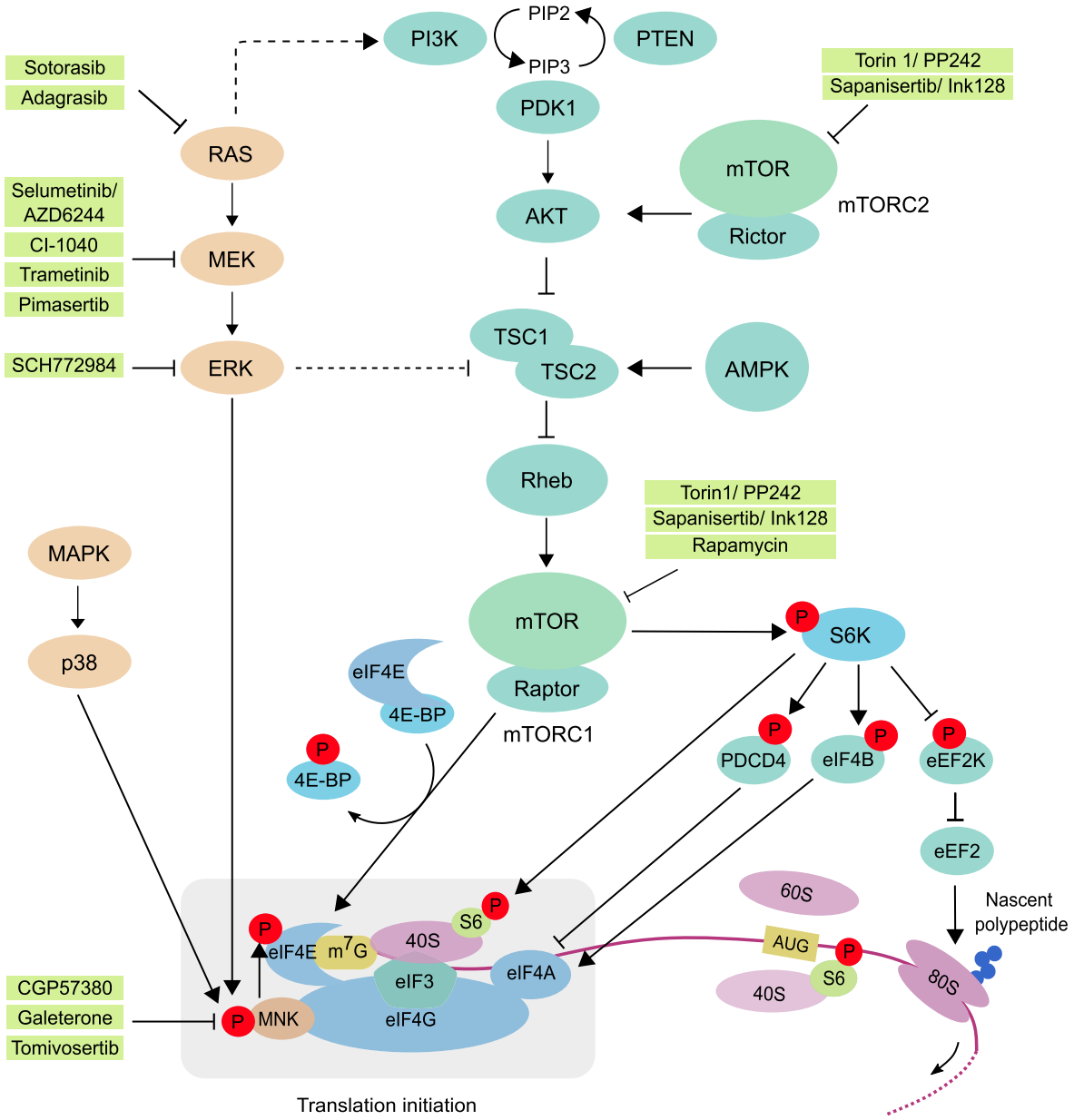

Figure 2. Upstream signaling pathways regulating translation initiation. The eIF4F complex is tightly regulated through PI3K/mTORC1 and MNKs. PI3K generates PIP3 leading to the phosphorylation of PDK1 and subsequently AKT. PIP3 can be reversed into PIP2 though PTEN phosphatase activity. AKT phosphorylates TSC1/2 to hinder their dimerization, inhibiting the Rheb GTPase which activates mTORC1. mTORC2 can also lead to subsequent activation of AKT. Upon energy deprivation, AMPK activates TSC1/2 dimerization, inhibiting mTORC1 activity to attenuate energy-consuming translation. 4E-BPs and p70S6Ks are substrates of mTORC1. Phosphorylation of 4E-BP releases eIF4E, allowing eIF4F formation. Phosphorylation of S6K leads to a phosphorylation cascade, including the elongation factor kinase eEF2K, the ribosomal protein S6, PDCD4 and eIF4B. The last two are an inhibitor and an activator of eIF4A, respectively. *KRAS* mutation leads to the activation of MNKs downstream of RAS/ERK and p38/MAPK pathways to regulate eIF4E through phosphorylation. Therapeutic agents under ongoing investigation, targeting these pathways, are noted in green boxes. MEK/ERK inhibitors are described in (54).

jections in mice), indicating that MNK1 kinase acts in acute pancreatitis via another substrate (47). In PDA cancer cells, eIF4E phosphorylation is induced in response to Gemcitabine treatment through the expression of specific MNK2 splice variants (48). Moreover, irradiation was also shown to induce eIF4E phosphorylation, leading to enhanced translation of Sox2 mRNA. In turn, the Sox2 transcriptional program favors repopulation after irradiation (49). Importantly, eIF4E phosphorylation was also associated with poor prognosis in PDA patients (48) as observed in other malignancies including melanoma or prostate cancer (44,50).

Targeting oncogenic pathways regulating translation in PDA

This section briefly summarizes the importance of targeting RAS/MAPK or PI3K/AKT/mTOR pathways in regard to PDA development and their impact on protein synthesis.

These pathways are crucial for PDA development. This was demonstrated in KC GEMMs by partial or complete inactivation of p110α PI3K, PDK1, MEK or ERK, which efficiently prevents RAS-driven tumors (51–53). Thus, a large panel of inhibitors targeting either MEK/ERK or PI3K/AKT/mTOR (see examples in Figure 2) has been developed, but showed limited efficacy in human subjects, in part due to toxicities (reviewed in 54). A glimmer of hope is now coming from KRAS G12C inhibitors [such as AMG510 (sotorasib) and MRTX849 (adagrasib)], and from a newly identified KRAS G12D inhibitor which could be administered to >30% of PDA patients (55).

Rapamycin, a first-generation and allosteric mTOR inhibitor, was shown to be efficient on p70S6K and 4E-BP1 dephosphorylation, and to arrest PDA cell growth *in vitro* (56). Unfortunately, Everolimus, a Rapamycin analog, showed minimal clinical activity in Gemcitabine-refractory patients and metastatic PDA (57). Nonetheless, Rapamycin analogs remain first-line treatment for metastatic neuroendocrine tumors. Thus, second-generation mTOR inhibitors, which are kinase inhibitors, were developed to circumvent the activation of the mTORC2 complex, consecutive to the Rapamycin-mediated inhibition of mTORC1 (58). Ink128 mTOR kinase inhibitor (also referred to as MLN0128 or Sapanisertib) has shown ability to reduce phosphorylation of mTORC1/2 target 4E-BP1 *in vitro* on PDA cancer cells, as well as on xenografts 6 h after treatment. In addition, Ink128 enhances sensitivity to radiotherapy through inhibition of cap-bound eIF4F formation *in vitro* and *in vivo* (59). Importantly, 4E-BP1 expression loss observed in PDA cells over-rides the protein synthesis suppression mediated by mTOR inhibition and led to increased resistance, independently of the mTOR inhibitors used in our studies (32,37). Aside from the involvement of mTOR in translational control, it is important to point out that mTOR inhibition could also favor autophagy induction and survival of cancer cells, especially under nutrient-poor conditions typical of the PDA microenvironment (60).

The development of MNK inhibitors, including CGP57380, Galeterone or EFT508/Tomivosertib, was thought to counteract the deleterious effect of eIF4E phosphorylation in cancer. CGP57380 was reported to favor Gemcitabine-induced apoptosis (48). Importantly, transient pharmacological or genetic inhibition of MNK was also reported to impact tumor phenotype, reducing the frequency of the mesenchymal phenotype, which is a known factor in chemoresistance (61). Galeterone and analogs have also shown similar properties *in vitro* and displayed a stronger antitumoral activity *in vivo* as compared with CGP57380. Galeterone reduced not only eIF4E phosphorylation but also MNK1/2 expression (62). Nonetheless, expression levels of 4E-BP1 should be considered when using MNK inhibitors. In fact, our work demonstrated that 4E-BP1 expression loss in PDA cells increased eIF4E phosphorylation, independently of MNK expression. In addition, disrupting the eIF4E–eIF4G interaction (which mimic 4E-BP1 action) can reduce eIF4E phosphorylation (32,63). The relevance of targeting eIF4E phosphorylation in the clinic will hopefully confirm these encouraging *in vitro* results. Clinical trials assessing the efficacy of MNK inhibitors such as Tomivosertib (NCT02605083) or Galeterone (NCT04098081) for the treatment of PDA as well as other solid tumors are currently ongoing.

TRANSLATION REGULATION BY THE INTEGRATED STRESS RESPONSE PATHWAY

During stress conditions, cancer cells differentially regulate the protein synthesis process in order to survive. This modulation occurs at the translation initiation step through the TC comprising eIF2-GTP and Met-tRNA$_i$ (see Figure 1), and is called the integrated stress response (ISR; see Figure 3).

Upon stress, eIF2α phosphorylation plays a key role in cell fate, triggering either a cell survival program or cell death. Activation of any of the four kinases PERK (Protein kinase R-like endoplasmic reticulum kinase), GCN2 (General control nonderepressible), HRI (Heme-regulated inhibitor) or PKR (protein kinase R) upon ER stress, amino acid deprivation, heme deficiency or viral infection, respectively, phosphorylates eIF2α. As a consequence, global cap-dependent translation is attenuated, while the translation of specific mRNAs is triggered following eIF2α phosphorylation (64). Most of these specific transcripts harbor at least one efficiently translated upstream open reading frame (uORF) that represses translation of the main coding ORF under normal conditions. The transcription factor ATF4 is currently the best-characterized factor induced in response to eIF2α phosphorylation, and it was shown to regulate expression of genes implicated in diverse stress responses. This mechanism of the ISR also includes the PERK-branch of the unfolded protein response (UPR) (65) which was shown to be importantly deregulated in many cancers including PDA, contributing to tumorigenesis and resistance.

ISR, a risk factor promoting PDA tumor development and survival

ISR was shown to be constituvely activated in PDA. The eIF2 kinase PERK, as well as eIF2α phosphorylation, and the protein chaperone GRP78 (BiP) were shown to be significantly increased in PDA compared with normal tissues, and to be associated with worse survival rates (66,67). In fact, experimental pancreatitis induced eIF2α phosphorylation (68). Moreover, inhibiting the ISR through PERK deletion (69,70) or ATF4 deletion (71) showed pancreatic damage induction. In addition, Salubrinal, an inhibitor of GADD34 and CReP (eIF2α phosphatases), was shown to favor pancreatitis in C57BL/6 mice by promoting the ISR (72), similarly to the PERK inhibitor GSK2606414 (73), showing that the ISR pathway is important for both pancreatic homeostasis and PDA development.

ISR implication in PDA adaptation to environmental stress

Pancreatic tumors are particularly exposed to high hypoxia and nutrient stress due to poor vascularization and a dense microenvironment (74). Therefore, PDA set up mechanisms to overcome these nutrient and oxygen deficiencies, especially through ISR-mediated transcriptional and metabolic reprogramming to allow adaptation to these conditions. Importantly, a tight regulation of eIF2α phosphorylation allows rapid protein synthesis recovery in PDA

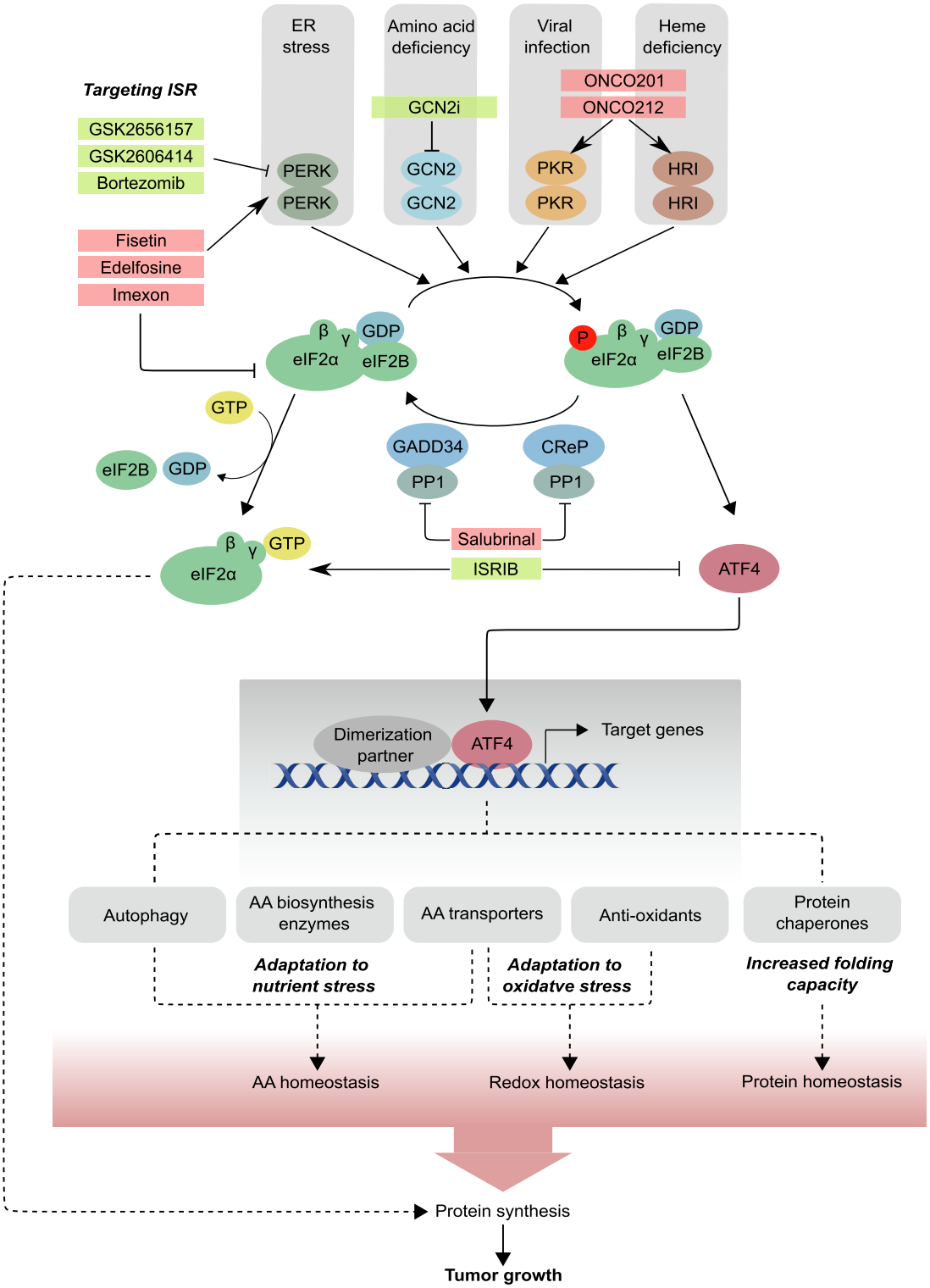

Figure 3. Integrated stress response. The ISR is a cellular response to different environmental stresses in order to promote cell adaptation and recovery. PERK, GCN2, HRI or PKR are activated upon endoplasmic reticulum (ER) stress, amino acid deprivation, heme deficiency or viral infection, respectively. Activation of any of these kinases induces the phosphorylation of eIF2 at the α-subunit. eIF2α can be dephosphorylated by two phosphatases, GADD34 and CReP, bound to PP1. eIF2α phosphorylation sequesters eIF2B, a guanine exchange factor which hinders the formation of the TC, attenuating the global cap-dependent translation initiation, and favoring the translation of a subset of mRNAs with several upstream open reading frames (uORFs) such as ATF4. The latter regulates the expression of a large panel of genes implicated in cell adaptation and survival, contributing to tumor growth. ISR-activating agents are noted in red boxes, while ISR inhibitors are noted in green boxes (105–107).

upon stress. Recently, overexpression of NUPR1 in PDA (75) was shown to play a crucial role in protein synthesis restoration through its interaction with eIF2α (76). This protein was shown to be implicated in the development of PDA as well (77).

Amino acid and redox homeostasis are highly regulated through constitutively active ISR in order to sustain tumor growth while increasing antioxidant defense (64). Among diverse effectors of an antioxidant response overexpressed in PDA, the X^{-}_{C} cysteine/glutamate exchanger (xCT) appears as a main regulator of redox homeostasis, as cysteine is a major intermediate for the production of the antioxidant glutathione (GSH). This transporter was shown to be up-regulated in PDA through ATF4 together with the ETS-1 transcription factor, activated downstream of the RAS–MEK pathway, which in turn contributes to RAS transformation by regulating the intracellular redox balance (78). Moreover, the transcription factor NRF2, a ROS regulator (which can be activated through phosphorylation by PERK), which protects components of the translation machinery against oxidation (29), was also found to be overexpressed in PDA (79).

To face nutrient deficiency, PDA cancer cells were reported to undergo a metabolic reprogramming orchestrated by KRAS through PI3K–AKT-mediated up-regulation of ATF4 mRNA and the GCN2/eIF2α/ATF4 axis (80). The cross-talk between the ISR and the nutrient-sensing pathways enables regulation of protein synthesis depending on the availability of energy and building blocks. ATF4 plays a central role in increasing nutrient availability through up-regulation of transporters such as LAT1, xCT, SLC1A4/5/7 or GLUT1 to favor amino acid and glucose uptake (81,82). ATF4 also controls expression of enzymes implicated in amino acid biosynthesis such as PHGDH, PSAT1, SHMT1 and ASNS for serine, glycine and asparagine biosynthesis, respectively (21,80,83). Sufficient availability of intracellular amino acids triggers protein synthesis by activating mTORC1 (84). Serine and glycine are crucial amino acids feeding one-carbon metabolism (85), responsible for nucleotide and glutathione production, and, together with asparagine, they have been reported to be essential for PDA tumorigenesis. Interestingly, depletion of asparagine synthetase (ASNS) combined with inhibition of AKT (80) or MAPK (83) pathways was shown to suppress tumor growth. Similarly, targeting ASNS in combination with a GCN2 inhibitor, GCN2i, was reported to induce P38MAPK which favors apoptosis (86). Serine dependency was also reported in specific PDA tumors lacking PHGDH. Despite the induction of ATF4 in the absence of serine, cells failed to induce PHGDH expression (87,88). This phenotype was associated with ribosome stalling on mRNAs enriched in specific Serine codons and selective translation of nerve growth factor mRNA (87). Our data reported a sustained ISR activation in Serine dependent PDA cells, allowing cell survival in the absence of Serine as well as a pronounced chemoresistance (88). Additionally, cellular mechanisms such as autophagy for macromolecule recycling have been reported to be regulated through ATF4 to increase intracellular nutrient availability (89). Therefore, PDA highly relies on the ISR and its effector ATF4 to adapt and sustain proliferation in a highly stressful microenvironment.

ISR implication in PDA chemoresistance

The ISR was shown to be implicated in PDA development by adapting to diverse stresses, including chemotherapies. The basal phosphorylation of eIF2α, as well as a low protein synthesis rate, were shown to inversely correlate with sensitivity to drugs such as Bortezomib, a proteasome inhibitor. Moreover, ATF4 is responsible for the expression of chemoresistance-related factors promoting rapid protein synthesis recovery in PDA such as NUPR1 (76), but also via increased antiapoptotic factors such as BEX2 and Bcl2a1 (90). PDA standard chemotherapies have been largely reported to induce the ISR, in contrast increasing its capacity to regulate protein and redox homeostasis (91,92). Interestingly, inhibition of HRI kinase sensitized PDA cancer cells to Bortezomib through apoptotic cell death and impaired translation inhibition (93). Furthermore, the UPR sensor BiP was shown to regulate the expression of NRF2 and the ATP-binding cassette (ABC) transporters, increasing chemoresistance. Silencing BiP sensitized PDA cell lines to diverse chemotherapies including Gemcitabine, Paclitaxel and 5FU, and reduced tumor growth through apoptosis in cell xenografts (92). As a result, modulating the ISR seems to be an interesting strategy to overcome chemoresistance.

Targeting the ISR as therapeutic potential in PDA

Combining chemotherapies with ISR-targeting agents appears an interesting therapeutic approach to prevent chemoresistance development (94). One of the most attractive strategies is to hinder the activation of the ISR to suppress the adaptive mechanisms in response to drugs. Targeting eIF2α kinases directly blocked the ISR and its adaptive response, leading to PDA tumor growth inhibition. PERK inhibitor GSK2656157 showed interesting pre-clinical results, but failed due to pancreatic and neuronal toxicity in patients (95–97). Similarly, Bortezomib was shown to inhibit PERK and to synergize with Cisplatin (98), but failed in a phase II clinical trial (NCT00416793) due to high toxicity. More recently, GCN2 inhibitor was shown to enhance the action of asparaginase, leading to apoptosis of resistant cancer cells (86). Interestingly, a recent phase II clinical trial (NCT02195180) showed increased PDA patient survival upon treatment with erythrocyte-encapsulated asparaginase in combination with either Gemcitabine or mFOLFOX (99). This highlights the potential of amino acid limitation as a therapeutic approach. On the other hand, to encounter high toxicity due to complete blockade of the ISR, ISRIB, a molecule partially hindering the ISR downstream eIF2α phosphorylation, has been developed (73). Mechanistically, ISRIB has been described (100) to increase the GEF activity of eIF2B, restoring sufficient protein synthesis for normal neuronal function to avoid toxicity, and decreasing ATF4 expression. Interestingly, alleviating the ISR through ISRIB also hindered induction of experimental pancreatitis (73), and decreased chemoresistance in combination with Gemcitabine *in vitro* and *in vivo* (90). Similar to observations made for PDA, reducing the ISR was shown to favor chemoresistance in other cancers including breast cancer, melanoma or acute myeloid leukemia (101–103).

Hence, although ISR activation has been demonstrated to promote cancer cell survival, prolonged activation of

eIF2α phosphorylation was shown to trigger stress-induced apoptosis leading to cell death. Indeed, overexpressing the eIF2α kinase PERK in PDA cell lines led to apoptotic cell death (104), as opposed to PERK inhibition. Moreover, different molecules that trigger the ISR, such as ER stress or oxidative stress inducers, showed inhibition of PDA progression by inducing apoptosis and autophagy (105–108). Recently, ONC212, an ISR activator, showed reduced PDA progression and a synergistic effect with chemotherapies (109). ONC201, a close analog of ONC212, also activated ATF4 through PKR and HRI, leading to increased expression of TRAIL and DR5 (110). Furthermore, many pharmacological molecules that target protein chaperones, such as HA15 and IT-139, were also shown to increase PDA sensitivity to standard chemotherapies. This includes resistance to Gemcitabine, Paclitaxel and 5FU *in vitro* and *in vivo* by inhibiting the protein folding capacity upon stress (92,111–113).

The ISR is at the center of cancer development, survival and resistance through a cross-talk between adaptation to stress and cell proliferation. Therefore, targeting this chemoprotective pathway seems to be a promising strategy in combination with standard chemotherapies. However, as the ISR may trigger apoptotic or survival fate in PDA cancer depending on the nature, intensity and duration of the stress, targeting this pathway without complementary cytotoxic drugs remains challenging. This dual role of the ISR is not limited to PDA but extends to many other cancers [for a review, see (94)].

DISCUSSION AND FUTURE DIRECTIONS

Active protein synthesis is considered as a hallmark of cancer cells. It is required for cellular growth and doubling of cellular organelles prior to division. Many alterations in translation regulatory processes have been described over the last decades in PDA, especially at the initiation step. PI3K/AKT/mTOR and RAS/MEK/ERK pathways are crucial in the control of protein synthesis. Therefore, targeting those upstream signals regulating translation initiation led to the development of a large panel of molecules inhibiting cancer progression in pre-clinical PDA mouse models including GEMMs, orthotopic grafts and PDX. However, among the few drugs that reached clinical trials, most failed to show higher efficacy than the current first-line chemotherapies. Therefore, patient stratification, based on specific tumor biomarkers, will be crucial to reveal the full potential of these pharmaceutical agents as personalized treatments for PDA patients (114).

Recent studies on targeting the ISR in combination with chemotherapies showed promising results. However, entirely suppressing the adaptive stress response pathway seems to enhance side effects on healthy tissues beside cancer cells, such as neurotoxicity. In fact, depleting PERK kinase or ATF4 was shown to highly impact pancreatic function (69,71). Moreover, ISRIB, which did not show any toxicity in mice, was shown to be only efficient within a defined window of stress activation (115), which renders its activity unpredictable in patients. Therefore, enhancing the ISR seems to be more adequate in combination with other chemotherapies. Along the same lines, ONC201, which induces the ISR, has shown encouraging results in patients with glioblastoma (116). Although the mechanism of shifting the ISR from stress adaptation to stress-induced cell death remains elusive, identifying other mRNAs translationally regulated by eIF2α phosphorylation is now urgent to understand the mechanism of ISR-mediated cell survival, as ATF4 was shown to regulate <40% of the ISR downstream genes in mouse embryonic fibroblasts (MEFs) (81). The ISR also regulates various cellular mechanisms such as autophagy, metabolism and immunity, which must be taken into account to avoid unwanted side effects and choose the best therapeutic combinations.

Among pharmaceutical targets of the protein synthesis apparatus, inhibitors of the translation elongation process remain rare or poorly efficient, apart from the only FDA-approved drug, Homoharringtonine (SynRibo). The methyltransferase METTL13, which targets eEF1A and enhances its activity, has been recently identified as essential for both protein synthesis and PDA growth (117). Pharmacological inhibitors remain to be developed to deepen the implication of METTL13 in PDA development and resistance. In addition, CG7, the inhibitor of eIF5A hypusination, and A484954, an inhibitor of eEF2K, have limited efficacy (millimolar range). Pharmacological improvement of these compounds might reveal the full therapeutic potential of targeting translation elongation in PDA cancer cells.

The PDA microenvironment represents a substantial proportion of the tumor volume (50–80%) where cancer-associated fibroblasts (CAFs) are the most abundant cells. CAFs have been described to support tumor growth through the massive secretion of extracellular matrix (ECM) as well as pro-inflammatory and chemo-protective cytokines (118). This important protein synthesis capacity has been poorly characterized despite some papers highlighting the interest in controlling mRNA translation in CAFs. The requirement of ATF4 for the massive type I collagen production supports that notion (119). Our group identified somatostatin analogs as a potential companion of chemotherapy in PDA. Somatostatin receptors are absent in PDA tumor cells but are expressed in CAFs. Mechanistically, we showed that a somatostatin analog, SOM230, could massively reduce protein synthesis via inhibition of the PI3K/AKT/mTOR/4E-BP1 axis. SOM230 diminishes interleukin-6 secretion by CAFs and blocks its pro-invasive and chemoprotective effect on cancer cells (120). Finally, combination of Gemcitabine with SOM230 blocks tumor growth and metastasis *in vivo* (121). These last examples illustrate one of the most important and active research axes on PDA, the stroma. Strategies to attack PDA tumor cells, such as chemotherapies or targeted therapies, have so far led to development of adaptive resistance mechanisms of tumor cells, as illustrated in this review. Essential crutches from the stroma further support the resistance mechanism. These include the presence of a dense ECM forming a physical barrier to drug delivery (122) and producing a hypoxic environment that selects the most plastic tumor cells, as well as CAFs and macrophages participating in chemotherapy titration or pro-survival cytokine secretion (123). Targeting translational control in PDA should encompass an integrative view of PDA biology and incorporate stroma weakness to make PDA tumors falter and finally fall.

LIMITATIONS

Several limitations apply to this review. Some relevant studies may have been missed although we aimed toward the most systematic review. Finally, due to space limitations or the existence of other valuable review articles, some sections were simplified. This includes the involvement of elongation factors in PDA (124) and targeting of the MAPK pathway in RAS-mutated cancer (54) which have been recently reviewed.

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