The role of the androgen receptor as a driver and mitigator of cellular stress

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Abstract

Prostate cancer is a high-incidence male cancer, which is dependent on the activity of a nuclear hormone receptor, the androgen receptor (AR). Since the AR is required for both normal prostate gland development and for prostate cancer progression, it is possible that prostate cancer evolves from perturbations in AR-dependent biological processes that sustain specialist glandular functions. The archetypal example of course is the use of PSA, an organ-type specific component of the normal prostate secretome, as a biomarker of prostate cancer. Furthermore, localised prostate cancer is characterised by a low proliferative index and a heterogenous array of somatic mutations aligned to a multifocal disease pattern. We and others have identified a number of biological processes that are AR-dependent and represent aberrations in significant glandular processes. Glands are characterised by high-rates of metabolic activity including protein synthesis supported by co-dependent processes such as glycosylation, organelle biogenesis and vesicle trafficking. Impairment in anabolic metabolism, protein folding and processing will inevitably impose proteotoxic and oxidative stress on glandular cells, and in particular,

luminal epithelial cells for which secretion is their primary function. As cancer develops there is also significant metabolic dysregulation including impaired negative feedback effects on glycolytic and anabolic activity under conditions of hypoxia and heightened protein synthesis due to dysregulated PI 3-kinase/mTOR activity. In this review we will focus on the components of the AR regulome that support cancer development as well as glandular functions focussing on the unfolded protein response and on regulators of mTOR activity.

The Prostate gland – dealing with a heavy workload.

The prostate gland is a highly metabolically active organ. Secretory luminal prostate cells within the gland are net citrate secretors to provide citrate as a metabolite, which supports ATP production to support sperm viability. In this sense the TCA cycle, which would otherwise oxidise citrate for aerobic respiration in other cell types, is impaired. Prostate cells in the untransformed healthy prostate are able to carry out this function due to their capacity to store zinc through the overexpression of the ZIP1 zinc transporter, which allows zinc accumulation to inhibit citrate oxidation (Eidelman et al., 2017). Prostatic tissue seems to be highly metabolically active and glycolytic in nature than other tissues as it tries to cope with a highly secretory output. This effect observed in untransformed prostates points to a basal ATP production mechanism dependent on glycolysis akin to a Warburg effect when compared to other tissues (Barfeld et al., 2014). Such secretory output and glycolytic activity require high protein demand and protein turnover and as such an increased protein folding capacity. Secretory proteins are indispensable in this process and their processing and folding occurs in the endoplasmic reticulum (ER). Proteins may be misfolded whilst assuming their tertiary and quaternary three-dimensional conformation and as such may be programmed for refolding or for terminal degradation. At any given point, the ER has a certain capacity for coping with misfolded proteins. If the amount of misfolded proteins exceed this threshold, a

physiological phenomenon occurs termed ER stress. There is a designated homeostatic mechanism designed to deal with misfolded protein induced ER stress called the Unfolded Protein Response (UPR). As its name suggests, this mechanism attempts to clear the ER of misfolded proteins through a few different functional outputs that include halting translation, increasing folding capacity and expanding the ER. The UPR is extremely important in the life cycle of a cell as it acts as a death or adaptation rheostat against adverse conditions. As such if the UPR is overwhelmed the cell is signalled for apoptosis (Almanza et al., 2018). Naturally this plays significant roles not only in normal physiology (B cell expansion, insulin production) but also in disease; a hyper-adaptive UPR confers high survival selectivity to the cells that possess it (such as cancer) whilst a constantly overwhelmed UPR leads to mass dysfunction and thus degeneration (such as loss of islets of Langerhans in diabetes) (Doultsinos et al., 2017).

The Androgen Receptor – a key player in prostate physiology

The androgen receptor (AR) is a cytoplasmic resident receptor that upon androgen binding regulates gene transcription through crosstalk with transcription factors, nuclear translocation and response element binding eliciting both genomic and non-genomic activities. It is tethered to the cytoplasm by filamin A and binding of androgens such as DHT induces conformational changes in the AR to form an activation function binding surface (Bennett et al., 2010). The AR is paramount to prostatic development. *In vivo* studies where the AR has been knocked out (ARKO mouse models) have shown that in its absence, mice are aprostatic whilst it has been shown that AR expression in the prostate is a late developmental event mostly associated with terminal prostatic morphogenesis (Heinlein and Chang, 2004). The AR, although fairly ubiquitous, is most highly expressed in luminal epithelial cells (Bonkhoff and Remberger, 1993) which carry out highly secretory specialised functions in producing markers such as the prostate specific antigen (PSA) that is the major diagnostic biomarker in prostate cancer (Hoffman, 2011). As a

transcription factor itself, it has a gene regulatory network aligned to the expression and function of proteins associated with this specialised differentiation function that comprises glycolytic and anabolic metabolism, glycosylation, calcium and lipid metabolism and protein folding either by direct binding or by proximity effects on neighbouring genes (Barfeld et al., 2014; Massie et al., 2011). This effect is recapitulated in localised prostate cancer (Gorlov et al., 2009). There is evidence then that as prostate cancer develops the AR takes on the role of a transcriptional regulator for other gene networks but can also maintain biological processes that are advantageous to cancer development. These include glycosylation, lipid turnover and stress-relieving adaptation and reprogramming mechanisms, in adverse conditions (hypoxia, glucose deprivation) elicited by the tumour microenvironment, such as the UPR (Cultrara et al., 2018). The AR is very much implicated in multiple metabolic disorder pathophysiologies. As far as metabolic malignancies are concerned both prostate and breast cancer (BCa) fall under the remit of hormone sensitive neoplasms. Indeed, there is certain overlap between the two disease states with the AR being shown to play a significant role in BCa development. It does this by inducing proliferation whilst in PCa although ADT is effective in over 80% of cases, patients with CRPC develop treatment resistance mechanisms that include non-canonical androgen production, AR gene and/or protein upregulation among others (Proverbs-Singh et al., 2015).

The UPR and the ER stress response

The UPR signals through three major transducers. PKR-like endoplasmic reticulum kinase (PERK), Activating Transcription Factor 6 (ATF6) and the most evolutionarily conserved across species, inositol requiring enzyme 1 (IRE1) (Figure 1). According to one model of UPR activity, the luminal domains of these three ER resident transducers, when inactive, are bound to UPR sensor BiP (or GRP78, HSP70). BiP, alongside protein disulphide isomerases PDI4-6, senses misfolded proteins and binds onto them,

releasing the three UPR transducers to assume their active state conformations (Bertolotti et al., 2000; Eletto et al., 2014). In the case of IRE1 and PERK, this involves auto-phosphorylation and oligomerisation whilst in the case of ATF6 it involves the unveiling of an ER export motif that allows its translocation to the Golgi. All three sensors give rise to transcription factors that signal to the nucleus to induce various processes that attempt to resolve ER stress such as cessation of translation and increased folding capacity (Figure 1).

IRE1 is dual enzyme, encompassing both serine/threonine kinase and endoribonuclease activity. When BiP dissociates from IRE1, it utilises its kinase to trans-autophosphorylate and oligomerise. This allows a conformational change that activates the RNase domain which has two distinct functional outputs as well as induce JNK and TRAF signalling (Cheng et al., 2014; Zhu et al., 2014). Firstly it unconventionally cleaves the mRNA of XBP1 to produce XBP1s, a transcription factor that signals to the nucleus to induce mechanisms designed to deal with ER stress (Lee et al., 2008). Secondly it degrades a distinct subset of mRNAs and miRNAs to either decrease the rates of subsequent protein synthesis or release the brakes in gene expression imposed by miRNAs on other targets through a process termed RIDD (Hollien et al., 2009) (Figure 1). IRE1 exists in two isoforms IRE1α (hereafter referred to as IRE1) and IRE1β (Imagawa et al., 2008). Although IRE1α is ubiquitously expressed, IRE1β is only found in mucosal epithelia in the gastrointestinal and pulmonary tracts (MB Martino, L Jones, B Brighton, C Ehre, L Abdulah, CW Davis, D Ron, WK O'Neal et al., 2013). Recently it was shown that IRE1β is negatively regulating IRE1α in a dominant negative manner as IRE1β lacks the trans-autophosphorylation and oligomerisation capacity that IRE1α needs to exert its RNase mediated influence on cell physiology (Grey et al., 2020).

PERK, when activated, phosphorylates eIF2α which blocks translation by inhibiting eukaryotic translation initiation factor 2B (eIF2B) activity and as such reduce protein load. This translation inhibition does not extend to activating transcription factor 4 (ATF4) which synergises with CHOP to promote terminal UPR activities that involve signalling for apoptosis (Flaherty et al., 2014) (Figure 1). Indeed PERK is not the only Eukaryotic Initiation Factor Associated Kinase that is involved in these

signalling pathways. Protein kinase R (PKR) affects NFκB as well as eIF2α and has been shown to have tumour suppressive functions impacting cell growth and proliferation (Garcia et al., 2006). Additionally, general control non-de-repressible 2 (GCN2) is an important sensor of the integrated stress response (ISR), which is a common adaptive homeostatic pathway with the phosphorylation of eIF2α at its epicentre (Pakos-Zebrucka et al., 2016). GCN2 positively regulates the induction of ATF4 translation having an effect not only in cell homeostasis but also through amino acid starvation sensing mechanisms control immunological responses and inflammation (Xia et al., 2018; Ye et al., 2010).

ATF6 (the least studied UPR transducer) after being translocated to the Golgi it is being cleaved and induces the production of transcription factor ATF6f which among other functionalities can induce the expression of IRE1 and CHOP to complement and enhance the activity of the other two UPR arms, collectively contributing to pathological processes such as oncogenicity (Dadey et al., 2016) (Figure 1).

The UPR through its transducers and downstream transcription factors activates certain ER stress related pathways such as ubiquitin–proteasome or lysosomal dependent protein degradation. These involve ER associated degradation (ERAD) and autophagy respectively (Kruse et al., 2006). XBP1s and ATF6 push ERAD to attempt to clear misfolded proteins from the ER by translocating them to the cytosol to be proteasomally degraded. ERAD signalling is complemented by autophagy. When accumulation of misfolded proteins overwhelms ERAD, autophagy is induced to deal with large molecule and organelle degradation through auto-phagosome sequestration which is then fused with the lysosome (Tasdemir et al., 2008). This pathway seems to be more eIF2α and JNK signalling dependent and as such contribute further to apoptotic cell death (Tcherpakov et al., 2009).

The extent to which these biologies are relevant to the survival and progression of cancer is closely linked to the metabolic and genomic dependencies of each disease state they are involved in.

Roles of UPR in cancer

144

145 The UPR shapes both normal- and patho-physiology of different cell types through its stress response
146 elements but it also has unconventional roles in disease progression. PCa is the commonest male cancer
147 and displays a specific dependence on androgen metabolism through the AR (Heinlein and Chang, 2004).
148 Inhibition of the AR by second or first generation anti-androgens is the standard of care in PCa with some
149 success; over 80% of patients respond to treatment.

150 AR expression and UPR activity have been correlated and this may well be due to potential interplay
151 between the two biochemical cascades, but also due to an accumulation of AR protein aggregates in the
152 cytoplasm. During mouse embryonic cell differentiation it was shown that BiP interacts with the AR and
153 is recruited into AR cytoplasmic aggregates to induce the UPR whilst at the same time AR overexpression
154 saw an exacerbated generalised ER stress response that promoted apoptosis with cells displaying a
155 differentiation phenotype also showing an overabundance of AR aggregates (Yang et al., 2013). Sensing
156 of misfolded proteins is carried out by chemical chaperones such as protein disulphide isomerases (PDIs).
157 One such dimeric protein, AGR2, is involved in intestinal inflammation and promotes the development
158 of inflammatory disorders when disrupted as it causes imbalances in the ER homeostatic
159 microenvironment through autophagy or secretion of AGR2 itself (Maurel et al., 2019). Its little known
160 paralogue AGR3, has also been shown to have pro-tumoral properties as it is involved in micro-
161 environmental signalling processes that impact Src mediated cancer cell migration and adhesion (Obacz
162 et al., 2019). Interestingly both AGR2 and AGR3 have been shown to be androgen responsive genes and
163 transcriptionally upregulated in prostate cancer cells possibly due to the observation that both AGR2 and
164 AGR3 sequences comprise binding sites for the androgen receptor on the distal promoter and intron
165 regions respectively (Bu et al., 2013) (Figure 2). It is therefore evident that AR and the UPR interact in
166 both a direct and indirect manner and that AR driven cancers such as PCa have a hyper-adaptive UPR to
167 deal with the stress.

The IRE1-XBP1 axis of the UPR is also particularly relevant to sustaining AR activity in prostate cancer. By genetic targeting XBP1 and IRE1 as well as the use of IRE1 small molecule inhibitors the IRE1-XBP1 axis has been shown to support tumorigenesis in pre-clinical models of prostate cancer. In the same study ADT was shown to downregulate the expression of IRE1-XBP1 target genes and enhance pro-apoptotic signalling through the PERK-ATF4 arm and downstream CHIP/DDIT3 (Storm et al., 2016). Collectively this suggests that IRE1/XBP1 biology is important in untransformed prostate glands and sustains prostate cancer development prior to perturbing AR signalling. In order to more specifically target UPR-dependent pro-survival factors it would be helpful to have a more refined view of the UPR-driven sub-biologies that are most required for AR-dependent tumorigenesis. Lessons can be learned from studies in other cancer types and model systems.

For example IRE1 has been shown to govern cytoskeleton remodelling and cell migration through interacting with filamin A, which acts as an interphase interactor between the dynamic actin cytoskeleton and the UPR (Urra et al., 2018). This pertains to probability of crosstalk between the UPR and many other biochemical pathways that may be targetable in a multitude of conditions (Hetz et al., 2013) that include a diverse repertoire of cancers. Indeed, tumours are constantly exposed to both intrinsic and extrinsic perturbants that include genomic instability, inflammation, immune cell invasion and nutrient deprivation. In addition they undergo stressful processes such as endothelial mesenchymal transition (EMT) and differentiation/reprogramming to induce cancer cell invasion and metastasis, angiogenesis and treatment resistance all of which have been associated with the utility of the UPR (Urra et al., 2016). Depending on the cancer type, the nature of the oncogenes involved and the treatment history of the cancer there can be differential dependencies on different UPR axes but also, importantly, on different subcomponents/sub-biologies regulated by those axes. XBP1 has been shown to promote triple negative breast cancer (TNBC) by promoting a hypoxia driven, HIF1α mediated pathophysiology associated with poor prognosis (X Chen et al., 2014) and IRE1 inhibition sensitised TNBC cells to paclitaxel (Logue et al., 2018). Conversely in glioblastoma (GBM) it was demonstrated that XBP1 signalling confers worse

prognosis than RIDD signalling in GBM patients and when taken into the context of tumour aggressiveness based on angiogenesis, invasion and immune infiltration these two functional outputs of IRE1 seemed to play a dual and even antagonistic role in tumour progression (Lhomond et al., 2018). The implication of this work is that the regulation of lineage differentiation represents an impertinent UPR-dependent process fundamental to understanding the outcome for cancers treated with UPR inhibitors. Determining the extent to which this is also true in prostate cancer and the mechanistic basis for it if it is true will be a vital component of future UPR research in this and other cancer types.

By contrast a much more established UPR-regulated 'sub-biology' is ERAD. ERAD has been positively linked with PCa tumorigenesis as it has been shown that ERAD components are practically non-existent in normal prostate epithelial lines whilst significantly upregulated in cancerous cells; an effect also observed in patient derived tissue. Interestingly, the androgen responsive ERAD component SVIP, was shown to be an endogenous ERAD inhibitor that is downregulated by androgen treatment; something not observed with almost any other components such as Ufd2a, Derlin1 and Npl4 (Erzurumlu and Ballar, 2017) (Figure 2).

ERAD itself can support the turnover of lipids, proteins and other 'metabolites'. We know that as prostate cancer develops, unlike other cancer types, there is a metabolic switch associated with enhanced oxidative metabolism. This correlates with the hyper-methylation of the ZIP1 transporter promoter leading to a zinc deficient phenotype in PCa thereby overcoming zinc-accumulation-mediated cytotoxicity (Renty B Franklin, 2014) (Figure 2). Additionally, there is enhanced TCA cycle activity, reduced citrate secretion, reduced polyamine secretion and also enhanced oxidative stress (Herroon et al., 2018) by transforming their energy production to be able to oxidise citrate in order to produce ATP through the Krebs cycle as an early event in prostatic transformation (Costello and Franklin, 2000; Cutruzzolà et al., 2017). Moreover there is deregulated lipid metabolism and increased lipid turnover, studies showing that there is an alternative fatty acid desaturation pathway to the established palmitate metabolism that dually contribute to cancer cell proliferation (Vriens et al., 2019). Fatty acid metabolism and fatty acid oxidation

(FAO) in particular have been singled out as pharmacological targets in PCa. CDK9 inhibition for example has been shown to lead to accumulation of acyl-carnitines, which, being FAO intermediates, induced acute metabolic stress. Blocking this pathway showed promise for synthetic lethality and further studies showing that ER stress inhibits FAO in the liver may provide other therapeutic avenues of synthetic lethality involving AR targeting, FAO targeting as well as UPR modulation (DeZwaan-McCabe et al., 2017) (Figure 2).

Here too it is clear that the greatest potential to exploit the UPR to treat cancers can only arise from an understanding of the co-dependency between the UPR and other important mediators of cancer progression, in this case lipid metabolism. In exceptionally poor prognosis cancers such as GBM developing a refined understanding of this will maximise outcomes for patients. Prostate cancer by contrast is characterised by a very good prognosis for the majority of localised cases and consequently having learnt more about these relationships the first translational opportunities will arise in end-stage disease, sometimes referred to as castrate-resistant prostate cancer (CRPC)

Based on this switch we would predict that UPR-dependent biologies including lipid droplet biogenesis, antioxidant prediction and lipidic ERAD would be necessary complementary homeostatic/pro-survival processes. This more refined understanding of UPR dependency is lacking in localised/untreated prostate cancer in part due to the lack of pre-clinical models of early-stage disease.

CRPC

Prostate cancer progression to CRPC occurs in a minority of diagnosed cases over a period of several years and often downstream of radical treatment and treatment with androgen deprivation therapy/anti-

androgens (Hussain et al., 2018). Unlike localised prostate cancer it shows many of the Hallmarks of other poor-prognosis cancer types including a high incidence of somatic mutations in classical drivers of tumorigenesis such as p53, Rb, c-Myc. As a result significant progress has been made in sub-typing CRPC according to these mutational profiles through initiatives such as StandUp2Cancer. Genomic drivers and mutations identified through these studies and others include PTEN-loss, p53/Rb loss and Myc amplification/overexpression (Grasso et al., 2012) (Figure 3). In addition hypoxic signalling, which in part is due to HIF1α activation, also supports the evolution of CRPC and hypoxic gene signatures can prognostic localised prostate cancers as can mutational profiles (Ranasinghe et al., 2013). PTEN and c-Myc are currently the best studied in the context of UPR activity in prostate cancer and it's helpful to summarise this.

PTEN-loss is an mTOR activator compounding AR signalling

PTEN-loss confers enhanced PI3k/Akt signalling and downstream mTOR activation (Edlind and Hsieh, 2014) which in turn elicits an increased protein load and subsequent homeostatic mechanisms designed to deal with it. The ER is exceptionally important in metabolic processes and mTOR is a signalling pathway paramount to various physiological processes including energy metabolism, autophagy, apoptosis, translation and inflammation. Studies suggest that the predominant role of mTOR signalling is to suppress autophagy dependent survival during terminal ER stress, whilst inhibition of this pathway has been shown to induce the UPR in models of sarcoma (Briggs et al., 2017; Kapuy et al., 2014). Specifically, it seems that mTOR signalling may regulate IRE1 signalling by modulating its endoribonuclease dynamic response through mitochondria-ER contact site remodelling dynamics, potentially taking advantage of ER membrane expansion and its utility in protein quality control travel

routes in and out of mitochondria (Hansen et al., 2018; Sanchez-Alvarez et al., 2017). Furthermore, it is a pathway that has been implicated in the development and maintenance of CRPC through the PI3K-AKT-mTOR signalling axis (Figure 3).

There is a direct link between this and the AR signalling cascade with a dynamic interplay between the two contributing to disease exacerbation. In fact hyper-activation of this pathway is sufficient to induce PCa formation with the involvement of mTORC1 and 2 being essential for this to occur *in vivo* (Edlind and Hsieh, 2014). It has though been shown that PI3K-AKT-mTOR is not only in interplay but also more dominant than the AR pathway in PCa cells. Inhibition of PI3K activated AR signalling but unexpectedly the resulting effects were anti-proliferative whilst at the same time the mTOR inhibitor rapamycin activated AR target genes synergistically with androgen (Kaarbø et al., 2010). mTORC2 in particular seems to balance AKT activation through its interplay with eIF2α eliciting responses from the PERK arm of the UPR. Phosphorylation of eIF2α at S51 guides cell fate decisions dependent on AKT (Rajesh et al., 2015). Inhibition of mTORC2 induces eIF2αS51P which in turn depends on the activation of AKT promoting survival during stress (Tenkerian et al., 2015). As such, there is a clear link between protein synthesis, the UPR and AR signalling in PCa that could be exploitable in sensitising CRPC tumours by synthetic lethality mechanisms (Figure 3). Indeed mTOR inhibitors have been implicated in CRPC as progression to CRPC from PCa may be a result of somatic changes to the PI3K-AKT-mTOR pathway. Unfortunately overall inhibitors proved to be inefficacious in treating CRPC with feedback mechanisms between AR signalling and PI3K signalling being potential reasons for this (Statz et al., 2017). Since ADT or mTOR inhibition are inadequate in CRPC as monotherapies, the addition of UPR modulation could provide a novel therapeutic adjuvant approach to established therapies. Synthetic lethality has been proposed in advanced PCa in the form of PARP inhibition due to data pointing to homologous recombination defects in such tumours. Upon ADT therapy PARP-mediated repair mechanisms are upregulated which could mean that inhibiting PARP alongside ADT would provide a genetic cell state unable to cope with DNA strand breaks (Asim et al., 2017). PTEN-loss is also supporting cholesteryl

ester/lipid droplet storage, which could act as a substrate reservoir for steroid hormone synthesis as such pointing to lipid accumulation as a prognostic factor of PCa (Figure 3). Indeed accumulation resulting from exogenous lipoprotein influx and cholesterol ester storage positively regulates PCa proliferation, invasion and growth (Yue et al., 2014).

PTEN and the AR/lipid metabolism is supported by UPR signalling

Advanced stage prostate cancer is predominantly found to present with bone metastasis and even after ADT remains androgen dependent. During these metastatic processes, Ca++ metabolism is of paramount importance and is mediated by serine threonine intracellular kinases such as CAMKK2. This protein was found to be significantly overexpressed in malignant prostate glands compared to normal epithelium and *in vivo* murine experiments showed that inhibiting CAMKK2 resulted in tumoral growth inhibition as a result of its action as an AR regulator and stabiliser as well as a migration mediator through AMPK and glycolysis via the AMPK-PFK pathway (Figure 2) (Dadwal et al., 2018). CAMKK2 has also been shown to promote prostate cancer independently of the AMPK pathway by affecting lipid metabolism. Indeed, loss of CAMKK2 reduced the expression of acetyl-coA carboxylase and fatty acid synthase (FASN) which accompanied by an activation of AMPK showed decreased cell growth rates through inhibition of de novo lipogenesis. FASN was prominently upregulated in PTEN-null mice but was downregulated when CAMKK2 was inhibited in both PTEN-positive and PTEN-null backgrounds (Penfold et al., 2018). As such it is evident that this calcium/calmodulin protein kinase has an effect on both lipid and calcium metabolism, is AR dependent and affects lipogenesis factors such as FASN whose inhibition have been shown to induce the UPR (Figure 3) (Little et al., 2007). Further evidence of metabolic crosstalk between AR physiology and the UPR comes in the form of macrophage activation. CAMKK2 downregulation is detrimental to macrophage migration, cytokine & chemokine release and bacterial phagocytosis

(Racioppi, 2013) whilst high IRE1 activity was shown to be a major factor in macrophage recruitment towards another solid tumour, Glioblastoma Multiform (GBM) (Lhomond et al., 2018). The lipidic link to prostate cancer metastasis to bone sites has been further explored in the context of the ER stress response through studies showing that osteotrophic prostate tumour cells upregulated the HO-1 oxidative stress marker whilst at the same time upregulating BiP and XBP1s through lipid uptake in adipocyte rich environments (Herroon et al., 2018) (Figure 3).

Myc amplification is supported by UPR signalling

Myc overexpression leads to an enhanced dependency on TCA cycle metabolites (Figure 3) which include the products of processes such as beta-oxidation of lipids and glutamine consumption (Goetzman and Prochownik, 2018). Studies trying to discern these as resistance mechanisms have unveiled the E2F cell cycle regulator as the androgen independent driver of tumour growth accompanied by increased N-Myc activity (Handle et al., 2019). Interestingly, N-Myc is very similar to c-myc in function and structure and both play a role in prostatic neoplasia however, c-myc, one of the most highly activated pathways in PCa, has been shown to be dependent on IRE1-XBP1 axis function. Specifically, XBP1s is required for the activation of the c-myc transcriptional program and there was co-localisation of c-myc and XBP1s in human prostate cancer specimens pertaining to a positive feedback loop between UPR signalling, myc signalling and prostate tumorigenesis (Sheng et al., 2019) (Figure 3).

Other drivers of CRPC include cytoskeletal AR/UPR-related changes and UPR-centric hypoxic responses.

P53/Rb loss induces the release of stress-induced checkpoint control and maintenance of cell cycling (p53/Rb loss) (Sharma et al., 2007). This has been implicated in prostatic metastasis through the regulation of the RHAMM motility receptor which stabilises f-actin polymerisation through ROCK signalling (Thangavel et al., 2017). Such tumorigenic effects on the cytoskeleton has been already been described to have direct impact both on UPR (Urra et al., 2018) and AR (Castoria et al., 2011) mediated metastasis through filamin A signalling (Figure 3).

Whilst there are a significant number of hypoxic gene signatures linked to CRPC and there is evidence in other cancer types of a direct relationship between HIF1α and XBP1, the interplay between hypoxia and the UPR in prostate cancer is largely unexplored. We know however that there is a significant interplay with the AR and that hypoxia and HIF1α have very significant impacts on metabolic function, which we would expect to be compensated for by changes in UPR activity. Chronic ADT administration in the presence of hypoxia actually induced adaptation to antiandrogen treatment via the increase of glucose 6 phosphate isomerase (GPI) brought about by AR inhibition. GPI restores glucose metabolism by favouring a hypoxic glycolytic activity that compounds adaptation to ADT. This interplay is further evidence of hypoxia and the AR controlling a metabolic switch to promote CRPC development (Geng et al., 2018). HIF itself plays a central metabolic role evidenced by an overexpression of both HIF1α and HIF2α in the hypothalamus involved in both weight gain functions, glucose uptake and energy expenditure (Gaspar and Velloso, 2018). HIFs are also involved in lipid metabolism as uptake of extracellular fatty acids and triglyceride synthesis are upregulated during hypoxia through PPARg activated by HIF1 which additionally mediates the expression of FABP3 and FABP4 in tumour cells (Mylonis et al., 2019).

Under hypoxic conditions in breast cancer, XBP1 induces miR-153 by binding onto the promoter of PTPRN which in turn directly inhibits the expression of HIF1α and subsequently reduces the secretion of VEGFA to modulate angiogenesis (Liang et al., 2018). It has also been reported that HIF1α levels are regulated by ER stress whilst apoptotic processes are impacted by hypoxia and ER stress in a temporal manner (early and late respectively) (Lõpez-Hernández et al., 2015). This could be of particular significance in metabolic

362 disorders as for slow growing tumours such as PCa, the exact therapeutic window and which pathways
363 to target during this window, are of great importance to the efficacy of the therapy. Apart from hypoxia
364 another event that miRNAs have been shown to be involved in, in the evolution of PCa, is resistance to
365 radiotherapy. Indeed miR-191 was found to be upregulated in PCa when compared to normal prostate
366 tissue and correlated positively with higher Gleason scores. It produced these effects by interacting with
367 retinoid x receptor alpha (RXRA) which is downregulated in PCa (Ray et al., 2020). Such involvement of
368 miRNA in AR physiology, UPR biology and PCa pathophysiology and treatment resistance points to both
369 prognostic, diagnostic and therapeutic value for future studies in elucidating further mechanisms affected
370 by non-coding RNAs.

371

372 **Current and future perspectives on targeting the UPR in prostate cancer**

373

374 It is evident that the UPR and the AR are so interconnected that they present a valid therapeutic dual
375 target in PCa and especially in CRPC where conventional ADT is having little to no effect. However,
376 certain barriers to such an approach remain that predominantly have to do with patient stratification.
377 After all, the target is to provide therapeutic solutions for a heterogenous group of patients by targeting
378 a fine tuning mechanism that always oscillates around a homeostatic balance. This could perhaps
379 dissuade from a “one size fits all” approach where all patients are treated with the same combination of
380 UPR/AR modulators. As such, biomarker development is key. Classifying cancers according to their UPR
381 status is incredibly tricky due to the availability of material that these markers are derived from; in GBM,
382 for example the hugely aggressive nature of the tumour and its anatomical location make repeat biopsies
383 impossible. This means that the transcriptional data derived from sequencing these tumours upon
384 resection provide only a small, albeit valuable, window into the life time of the tumour and as such only
385 give a crystallised in time picture of UPR status. A status that as discussed earlier in this review could

potentially change during the temporal disease progression. Despite this drawback, some work has been carried out to generate activity signatures (to be distinguished from expression levels) of IRE1, XBP1s and RIDD function by combining data mining and gene ontology enrichment strategies. These approaches were able to classify GBM patients according to their IRE1 status as well as their XBP1/RIDD status in four distinct groups showing that IRE1 and XBP1 in particular is a tumorigenicity driver in GBM (Lhomond et al., 2018). Being able to distinguish between patients according to UPR status is of great importance considering the diverse role that the UPR plays in cancer physiology. As the UPR has been implicated in cancer stem biology (Peñaranda-Fajardo et al., 2019) it is possible that targeting the UPR in certain patients (depending on their UPR status) could affect cell populations that may be deleterious as, for example, a stem phenotypic change could lead to a more adaptive subpopulation of cells that promotes disease recurrence.

The advantage of studying prostate cancer in this instance is that the disease progression is relatively long with ample opportunity for sample collection including healthy tissue, malignant tissue, blood and urine. Since there are a plethora of biological materials to work with one question that needs to be addressed is whether looking for a transcriptional UPR classification would be the most clinically relevant. Moreover, even if a proteomic approach is preferred due to its translational value in the clinic would it be pertinent to select single protein biomarkers or is this a biology that truly requires a systems level approach? Considering that it is a complex biology with many upstream and downstream interactors, it seems that a systems approach is one of the most value. Of course, a caveat in this approach would require exceptionally well-annotated sample collections and models that represent the full spectrum of disease and diverse temporal disease evolution. There have been few attempts to classify prostate cancer using multi-parametric profiling comparing proteomic, transcriptomic and genomic approaches. Interestingly the conclusion of such a study was that EIF2/translational effects are more significant than previously appreciated when proteomics is performed as opposed to genomics or transcriptomics (Latonen et al., 2018).

Alternatively a systems level approach may not be the ultimate destination but rather if it were possible to undertake a much careful functional analysis of the key proteins and transcripts that are dependent on UPR activation and confer pro-survival biologies a robust set of activity biomarkers could be discovered alongside more selective and effective druggable targets. This has been the route taken to attempt to improve responses to AR-targeted therapies and improve the classification of prostate cancers moving beyond PSA as a marker (Eskra et al., 2019). Discerning UPR biomarkers poses a similar problem although on a greater scale because it has to address the potentially intersecting activities of a number of transcription factors which include the AR and XBP1/ATF6/ATF4 as well as the downstream transcription factors under their influence. Given that complexity, it is increasingly attractive to investigate the UPR alongside the AR and try to identify major players at the intersection of these major signalling pathways. This may have wide reaching implications given that also in other cancer types such as TNBC known AR targets like CAMKK2 and major pathways like myc are XBP1-HIF1α dependent (Casciano et al., 2020; Xi Chen et al., 2014). These transcription factors all require translocation/trafficking to control their activity and many require phosphorylation. These are potential biological processes in which to expect convergence on a small number of conserved regulatory factors that may be targetable and both members of the UPR and the AR itself are linked to the normal function of the cytoskeleton (Castoria et al., 2011; Urra et al., 2018).

Conclusions

CRPC is a major endocrinological malignancy with a significant public health burden. Although ADT treatments are effective in the vast majority of people with localised PCa, CRPC tumours display treatment resistance and metastatic characteristics; mechanisms shown to be governed by UPR activity; all the while maintaining an active AR physiology. So far, the literature has shown that the UPR is an

attractive druggable target in multiple malignancies and a complex biochemical response that has significant overlap with AR physiology. As such, it is logical to deduce that UPR modulation is a significantly attractive therapeutic avenue in sensitising ADT resistant tumours to either conventional or novel therapeutic regimes. Although UPR modulation has shown significant anti-tumoral effects in various cancer models including AR dependent ones such as PCa and breast cancer (Logue et al., 2018; Nguyen et al., 2018; Sheng et al., 2019) significant work has to occur for these results to be translatable in the clinic for the benefit of hugely heterogenous subject populations that are cancer patients.

Major considerations that may have to be addressed, before either dismissing or embracing the use of UPR targeting modulators (figure 4), include off target effects and the ubiquitous nature of this homeostatic mechanism. Furthermore, the balancing act that the three transducers of the UPR continuously carry out which is a contributing factor to why ER stress modulators (inducers or inhibitors) may have failed at clinical trial level (Almanza et al., 2018). As discussed earlier in this review, different arms of the ER stress response may play more or less significant roles during disease evolution. As such, a robust, informed set of data and prognostic, diagnostic and bridging biomarkers are required to guide which patients and at what exact therapeutic window would benefit from targeting key functional pillars of AR and UPR physiology in order to maximise positive clinical output. The crux of this metabolic crosstalk will be to discern whether the AR-UPR synergy is reciprocal or convergent. If reciprocal then UPR modulation could offer a route of sensitisation of CRPC to androgen therapies. If convergent, combinatorial targeting may well provide a more sustained response as, since multiple pathways are targeted, a synthetic lethality effect may be elicited.

Declaration of interest

The authors declare that they have no conflicts of interest in regard to any of the content of this article.

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759 **Figure Legends**

Figure 1: *The Unfolded Protein Response.* The three main UPR transducers IRE1, PERK and ATF6 signal through transcription factors XBP1s, ATF4 and ATF6 respectively to the nucleus to initiate responses to deal with misfolded protein induced ER stress. The interconnected nature of the three transducers is illustrated. Both IRE1 and PERK are implicated in the UPR associated NFkB pathway, ATF6 and XBP1s are reciprocally regulated and ATF4-induced CHOP impacts ATF6 signalling. In addition, IRE1 is essential for TRAF-2 and JNK signalling (Rothe et al., 1995; Urano et al., 2000). In the presence of genetic and physiological stress drivers, a physiologically normal cell would be overwhelmed and signal for apoptosis. Cancer cells utilise a hyper-adaptive UPR to survive.

Figure 2: *The Androgen Receptor in hormone sensitive prostate cancer.* Metabolic effect of AR genomic signalling on prostate cancer early development. Green and red arrows and letters respectively, represent genes and pathways upregulated or downregulated by the AR. AGR2/3, FASN and CAMKK2 induction lead to migration through Src signalling, lipid metabolism through FAO and glycolysis through citrate production respectively. Conversely, the downregulation of ERAD inhibitor SVIP leads to ERAD induction whilst the downregulation of zinc transporter ZIP1 reduces intracellular zinc accumulation and toxicity. Associated mechanisms include NFkB and JNK pathways (Xu and Hu, 2020; Zhang et al., 2009). These mechanisms aid prostate cancer cells to overcome metabolic stress and proliferate.

Figure 3: *AR-UPR-oncogenic driver crosstalk in CRPC.* Myc amplification (green) and PTEN, p53 loss (red) drive central mechanisms of CRPC development including a) glycolysis and oxidative stress; b) cytoskeletal organisation and metastasis; c) lipid metabolism and d) cell growth, proliferation and autophagy. The UPR (purple) is involved in all four mechanisms as well as inducing c-Myc itself, compounding PCa development. In parallel, AR signalling (mustard) drives UPR signalling whilst also

being involved separately in all four wide mechanisms driving PCa growth. Evidence derived from such studies point to a synergistic relationship between oncogenic drivers of CRPC, the AR and the UPR. The nature of this synergy be it reciprocal or convergent remains to be elucidated.

Figure 4: *UPR pharmacological targeting and oncogenic driver crossover in CRPC*. Non exhaustive list of modulators targeting each of the three major transducers of the UPR mapping onto the pro-survival processes driven by CRPC oncogenic drivers and supported by the UPR. PERK inhibition (dark blue): GSK2656157 has been shown to decrease transmissible ER stress affecting metastasis while ISRIB mediated PERK inhibition upregulates translation compounding anti-oncogenic misfolded protein mediated stress (Guthrie et al., 2016; Rodvold et al., 2017). IRE1 inhibition (Red): KIRAs have been shown to decrease fibrosis affecting motility (Thamsen et al., 2019), sunitinib has been investigated in clinical trials as an anti-angiogen ("A Phase II/III Study of High-dose, Intermittent Sunitinib in Patients With Recurrent Glioblastoma Multiforme - Full Text View - ClinicalTrials.gov," n.d.; Jha et al., 2011) whilst 4μ8C impacts autophagy by decreasing ERAD components (Erzurumlu and Ballar, 2017). MKC8866 was shown to block c-Myc signalling (Sheng et al., 2019) and toyocamycin lead to an upregulation of ROS formation impacting survival (Park et al., 2017) in prostate cancer models. ATF6 inhibition (Light blue): Ceapins were found to upregulate lipid droplet formation (Gallagher and Walter, 2016) whilst melatonin was shown to block ATF6 as well as decreasing ATP production impacting metabolism (Hevia et al., 2017). These investigations are supported by ongoing studies pinpointing the value of targeting UPR activity as therapeutic interventions in PCa such as targeting the splicing machinery affecting XBP1 signalling (Jiménez-Vacas et al., 2020).

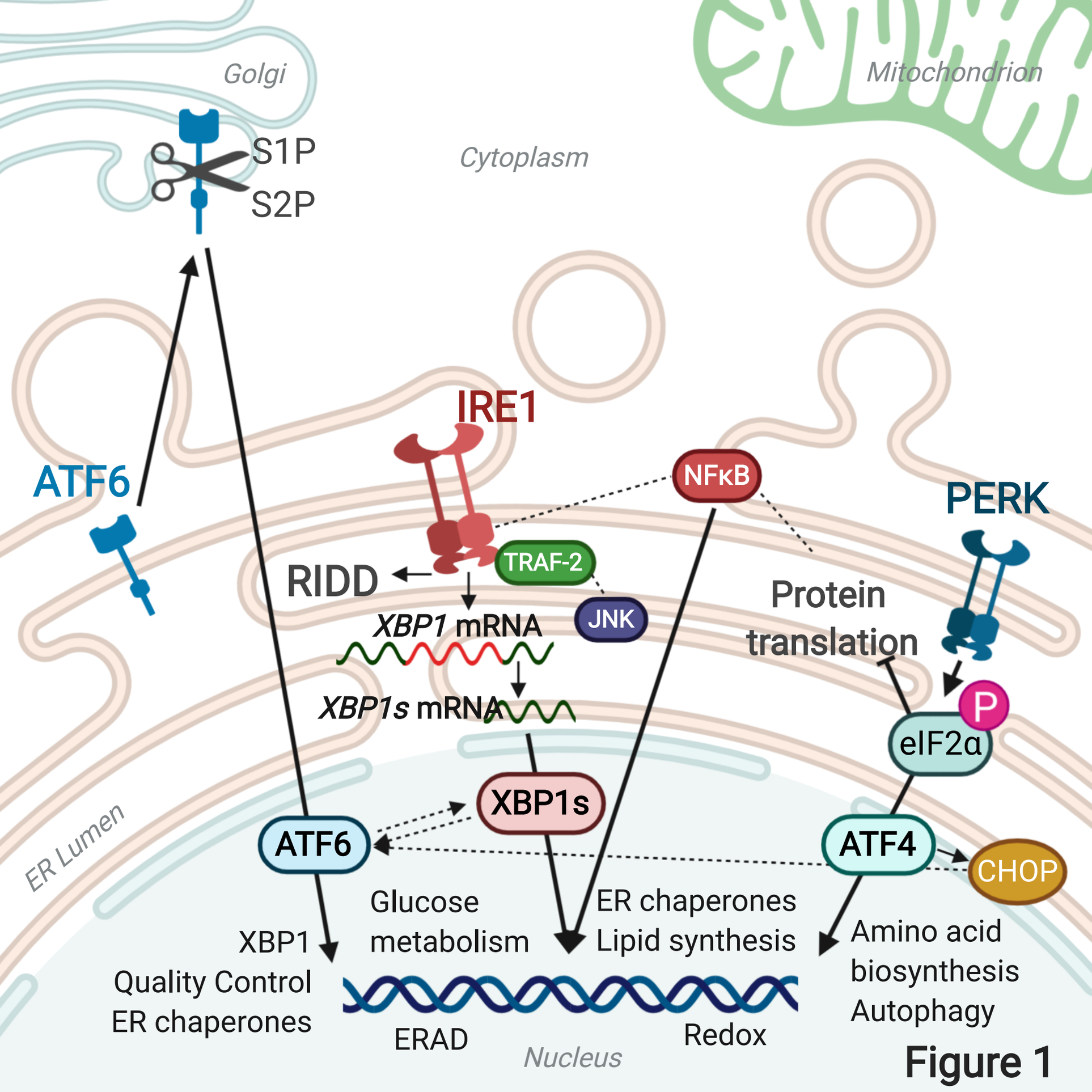
Golgi
Mitochondrion
S1P
S2P
Cytoplasm
IRE1
ATF6
NFκB
PERK
TRAF-2
RIDD
JNK
XBP1 mRNA
XBP1s mRNA
Protein translation
P
eIF2α
XBP1s
ATF6
ATF4
CHOP
ER Lumen
Glucose metabolism
ER chaperones Lipid synthesis
XBP1 Quality Control ER chaperones
Amino acid biosynthesis Autophagy
ERAD
Redox
Nucleus

Figure 1

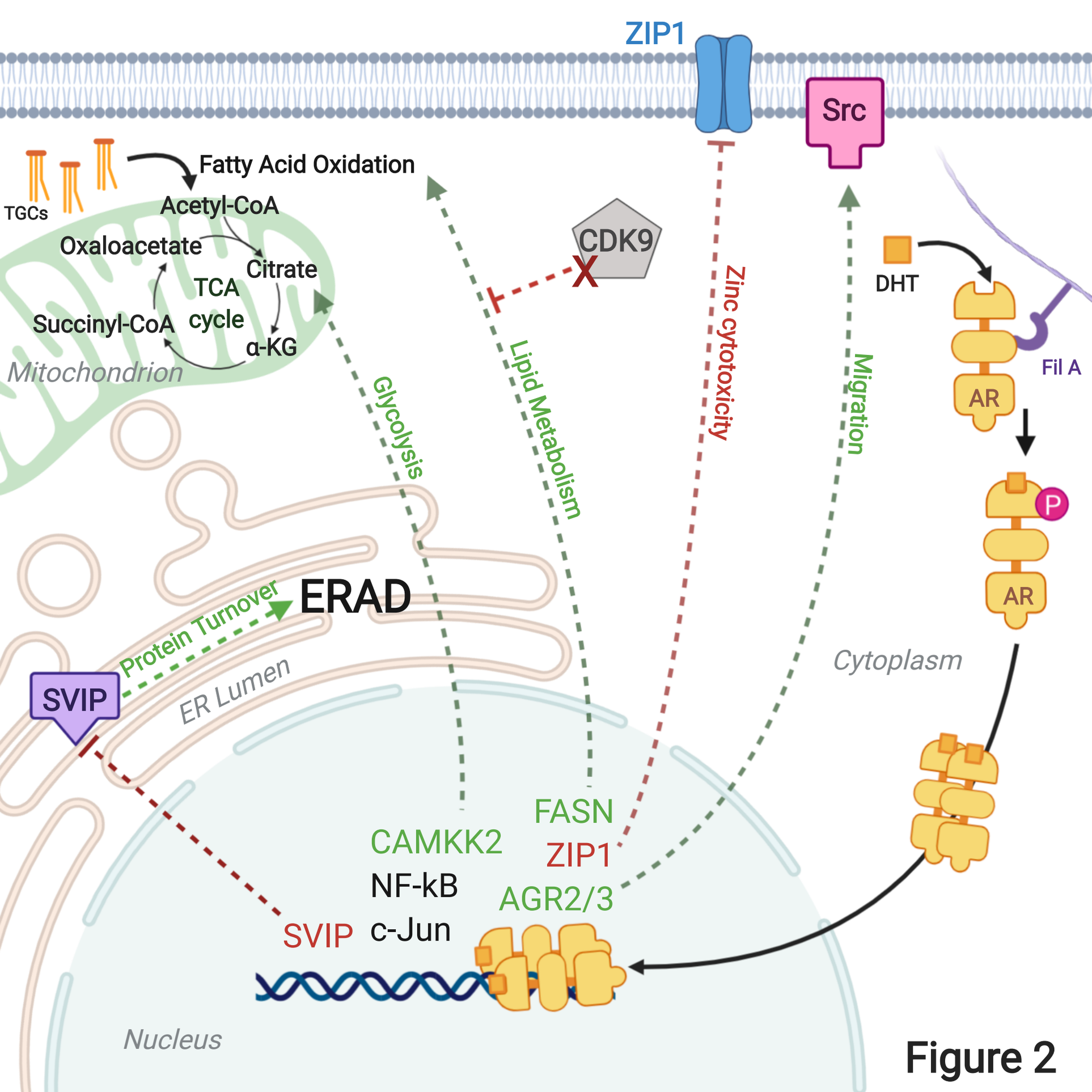
ZIP1
Src
TGCs
Fatty Acid Oxidation
Acetyl-CoA
Oxaloacetate
Citrate
TCA
cycle
α-KG
Succinyl-CoA
Mitochondrion
CDK9
Glycolysis
Lipid Metabolism
Zinc cytotoxicity
Migration
DHT
AR
Fil A
P
AR
Cytoplasm
ERAD
Protein Turnover
SVIP
ER Lumen
FASN
CAMKK2
ZIP1
NF-kB
AGR2/3
SVIP
c-Jun
Nucleus

Figure 2

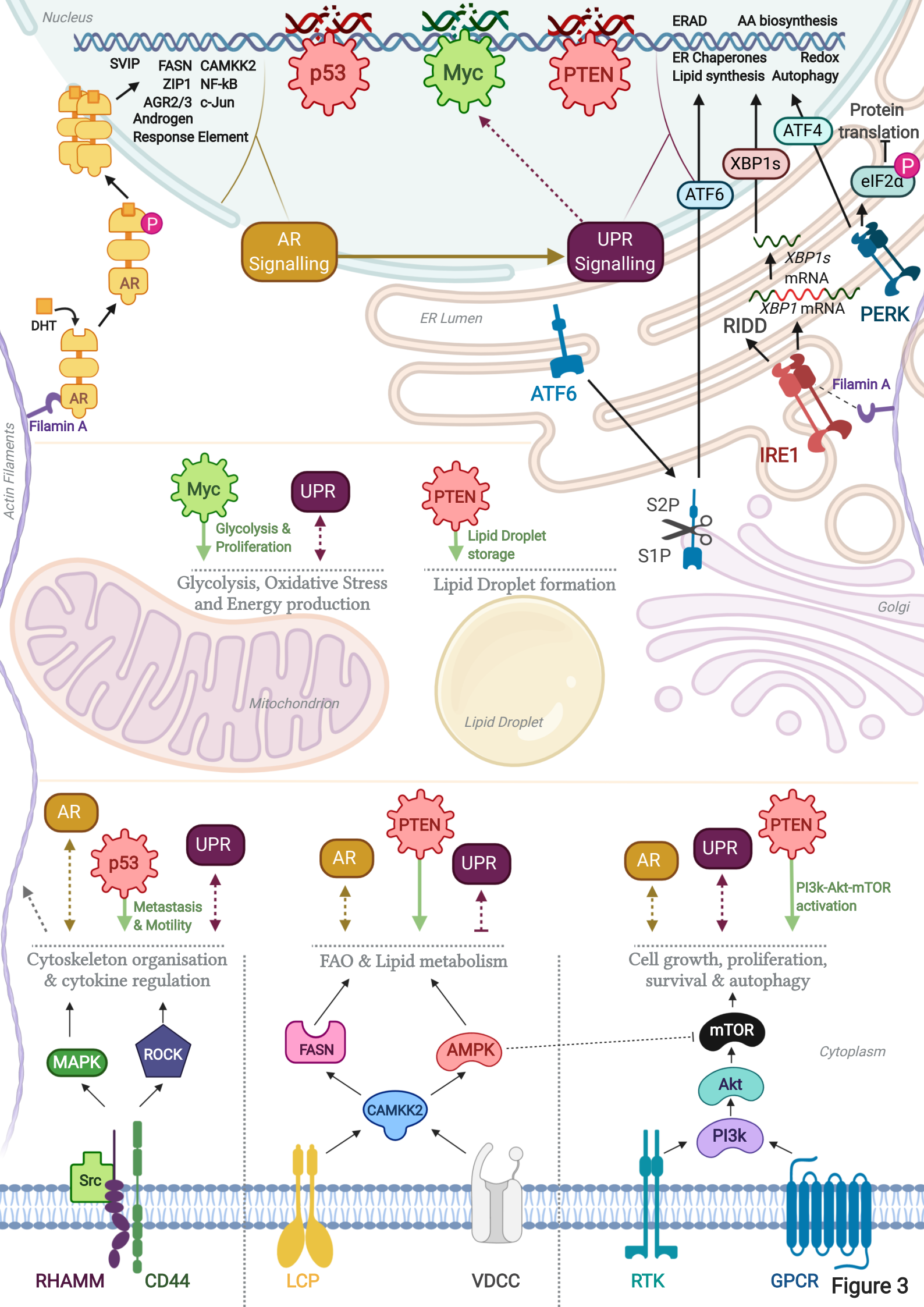
Nucleus
p53
Myc
PTEN
ERAD
AA biosynthesis
ER Chaperones
Redox
Lipid synthesis
Autophagy
SVIP
FASN
CAMKK2
ZIP1
NF-kB
AGR2/3
c-Jun
Androgen
Response Element
ATF4
Protein
translation
XBP1s
eIF2α
P
ATF6
AR
Signalling
UPR
Signalling
XBP1s
mRNA
XBP1 mRNA
PERK
RIDD
DHT
AR
ER Lumen
ATF6
Filamin A
Filamin A
IRE1
Actin Filaments
Myc
UPR
Glycolysis &
Proliferation
PTEN
Lipid Droplet
storage
S2P
S1P
Glycolysis, Oxidative Stress
and Energy production
Lipid Droplet formation
Golgi
Mitochondrion
Lipid Droplet
AR
p53
UPR
Metastasis
& Motility
AR
PTEN
UPR
AR
UPR
PTEN
PI3k-Akt-mTOR
activation
Cytoskeleton organisation
& cytokine regulation
FAO & Lipid metabolism
Cell growth, proliferation,
survival & autophagy
MAPK
ROCK
FASN
AMPK
mTOR
Cytoplasm
CAMKK2
Akt
PI3k
Src
RHAMM
CD44
LCP
VDCC
RTK
GPCR

Figure 3

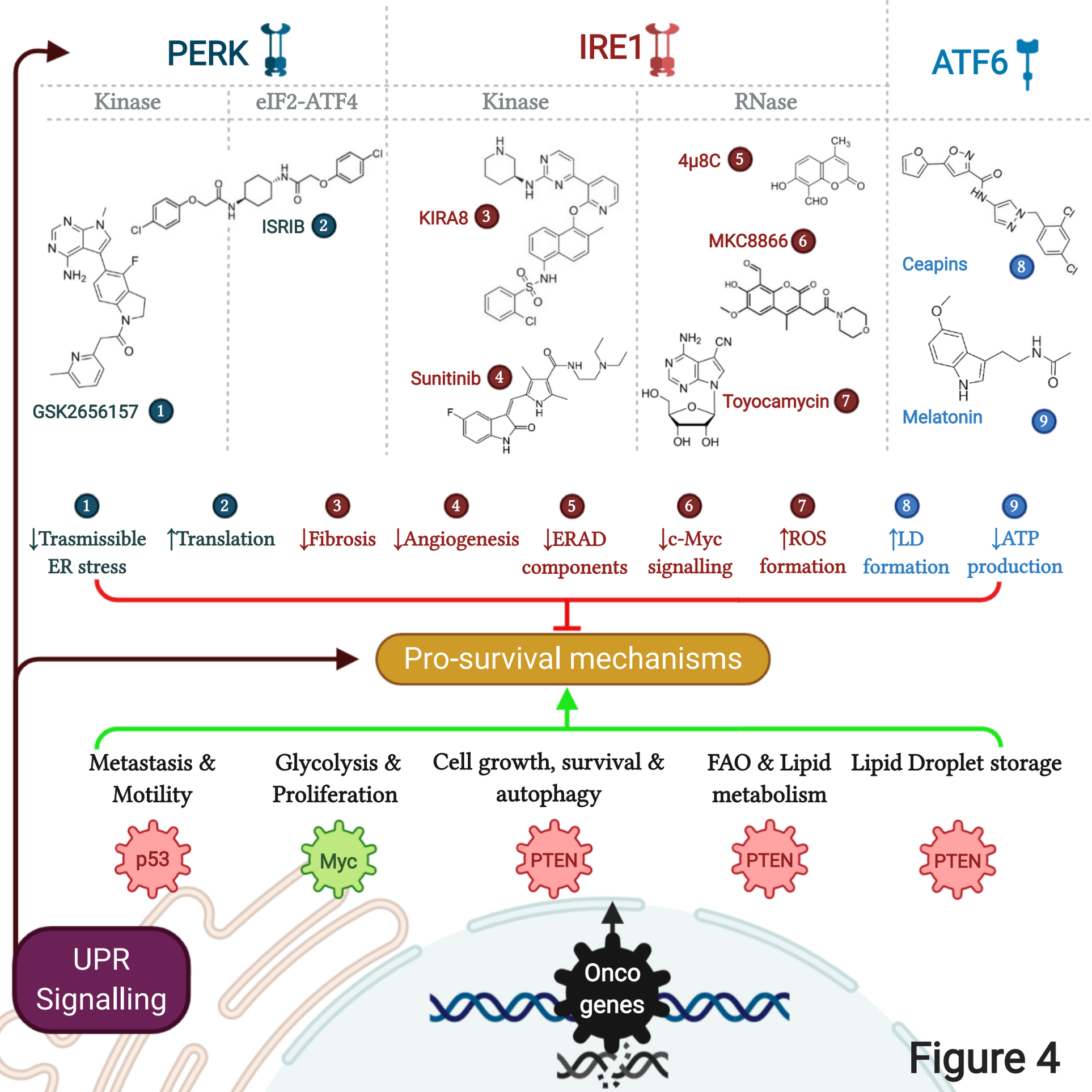
PERK
IRE1
ATF6
Kinase
eIF2-ATF4
Kinase
RNase
GSK2656157 1
ISRIB 2
KIRA8 3
Sunitinib 4
4μ8C 5
MKC8866 6
Toyocamycin 7
Ceapins 8
Melatonin 9
1 ↓Trasmissible ER stress
2 ↑Translation
3 ↓Fibrosis
4 ↓Angiogenesis
5 ↓ERAD components
6 ↓c-Myc signalling
7 ↑ROS formation
8 ↑LD formation
9 ↓ATP production
Pro-survival mechanisms
Metastasis & Motility
Glycolysis & Proliferation
Cell growth, survival & autophagy
FAO & Lipid metabolism
Lipid Droplet storage
p53
Myc
PTEN
PTEN
PTEN
UPR Signalling
Onco genes

Figure 4