

Minireview

Post-Transcriptional and Epigenetic Regulation of Estrogen Signaling

Andrea Cignarella, Carlotta Boscaro, Mattia Albiero, Chiara Bolego,¹ and Matthias Barton¹

Departments of Medicine (A.C., Ca.B., M.A.) and Pharmaceutical and Pharmacological Sciences (Ch.B.), University of Padova, Padova, Italy; and Molecular Internal Medicine, University of Zürich and Andreas Grüntzig Foundation, Zürich, Switzerland (M.B.)

Received February 7, 2023; accepted June 16, 2023

ABSTRACT

Post-translational and epigenetic regulation are important mechanisms controlling functions of genes and proteins. Although the “classic” estrogen receptors (ERs) have been acknowledged to function in mediating estrogen effects via transcriptional mechanisms, estrogenic agents modulate the turnover of several proteins via post-transcriptional and post-translational pathways including epigenetics. For instance, the metabolic and angiogenic action of G-protein coupled estrogen receptor (GPER) in vascular endothelial cells has been recently elucidated. By interacting with GPER, 17 β -estradiol and the GPER agonist G1 enhance endothelial stability of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) and capillary tube formation by increasing ubiquitin-specific peptidase 19 levels, thereby reducing PFKFB3 ubiquitination and proteasomal degradation. In addition to ligands, the functional expression and trafficking of ERs can be modulated by post-translational modification, including palmitoylation. MicroRNAs (miRNAs), the most abundant form of endogenous small RNAs in humans, regulate multiple target genes and are at the center of the multi-target regulatory network. This review also discusses the emerging evidence of how

miRNAs affect glycolytic metabolism in cancer, as well as their regulation by estrogens. Restoring dysregulated miRNA expression represents a promising strategy to counteract the progression of cancer and other disease conditions. Accordingly, estrogen post-transcriptional regulatory and epigenetic mechanisms represent novel targets for pharmacological and nonpharmacological intervention for the treatment and prevention of hormone-sensitive noncommunicable diseases, including estrogen-sensitive cancers of the reproductive system in women.

SIGNIFICANCE STATEMENT

The effects of estrogen are mediated by several mechanisms that are not limited to the transcriptional regulation of target genes. Slowing down the turnover of master regulators of metabolism by estrogens allows cells to rapidly adapt to environmental cues. Identification of estrogen-targeted microRNAs may lead to the development of novel RNA therapeutics that disrupt pathological angiogenesis in estrogen-dependent cancers.

Introduction

Steroid hormones such as estrogen interact with specific receptors in target tissues that mediate biologic responses affecting different cellular functions such as metabolism, proliferation, ion transport, excitability, and contraction (Deroo and Korach, 2006). In general, estrogen responses that are dependent on latent (hours/days) nuclear and transcriptional events are genomic responses and involve, among others, “classic” nuclear estrogen receptors (Hewitt and Korach, 2018). Nongenomic, rapid responses (seconds/minutes) do not require nuclear transcriptional events

and involve cytosolic signaling (i.e., protein kinases, cAMP, pH, Ca²⁺) downstream interactions of hormone with estrogen receptors expressed at the plasma membrane (Levin and Hammes, 2016).

Many of the genomic effects of estrogen are largely mediated through its binding to estrogen receptors (ER) ER α and ER β isoforms, members of the nuclear receptor superfamily (Alexander et al., 2021). ER α and ER β display distinct patterns of expression and function in various tissues (Matthews and Gustafsson, 2003). They both act as ligand-dependent transcription factors either by binding to the estrogen-responsive element with the consensus palindromic repeat, or by interacting with other transcription factors and by recruitment of cell-specific coregulators (Hall and McDonnell, 2005). Transmembrane estrogen receptors such as G protein-coupled estrogen receptor (GPER) have been shown to mediate nongenomic actions of estrogen in several tissues (Barton et al., 2018) as well as chronic (genomic) effects

This work was supported by the Swiss National Science Foundation [Grants 108 258 and 122 504] (to M.B.).

¹C.B. and M.B. contributed equally to this work.

M.B. is an inventor on US patent numbers 10,251,870, 10,682,341, and 10,980,785 for the therapeutic use of compounds targeting GPER.

dx.doi.org/10.1124/jpet.123.001613.

ABBREVIATIONS: AGO2, Argonaute 2; ER, estrogen receptor; E2, estradiol; GLUT1, glucose transporter 1; GPER, G protein-coupled estrogen receptor; miRNA, microRNA; PFKFB3, 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 3; SERM, selective ER modulator; USP19, ubiquitin-specific peptidase 19; UTR, untranslated region.

(Meyer et al., 2016; Barton et al., 2018). In extra-nuclear-initiated pathways, ER α , ER β , and GPER activate rapid kinase signaling on ligand binding (Bologa et al., 2006). Regulation of gene transcription by GPER involves mitogen-activated protein kinase pathways and regulation of transcription factors (Prossnitz and Barton, 2023). The clinical and translational aspects of rapid nongenomic responses to estrogen (and the resulting downstream chronic effects) mediate estrogen signaling in a variety of conditions such as breast cancer, cardiovascular, and neurodegenerative diseases (Mauvais-Jarvis et al., 2022; Prossnitz and Barton, 2023). Of note, rapid responses have been recently reported to determine sexual dimorphism of estrogen actions in hormone-producing, nonreproductive issues such as the adrenal gland (Carocchia et al., 2016).

In addition to ligands, the activities of ERs can be modulated by post-translational modifications. ER α , for example, is subject to phosphorylation, ubiquitylation, sumoylation, and acetylation (Anbalagan et al., 2012). This will be further described in a separate section below. mRNA decay and translation, together with transcription, determine the abundance of cellular RNA and protein (Hu and Collier, 2012). Additional post-transcriptional mechanisms found in eukaryotes include the regulation of splicing and polyadenylation (Schaefer et al., 2018). The post-translational changes of components involved in the signal transduction cascades, typically initiated by the binding of a ligand to its receptor, are strong indicators of the cell response in which it occurs (Benayoun and Veitia, 2009). Acetylation and deacetylation of a lysine residue on proteins are among the most common post-translational modifications that act as molecular switches in regulating important cellular events (Drazic et al., 2016). The lysine residue is reversibly modified through acetylation and deacetylation by lysine acetyltransferases and lysine deacetylases, respectively (Haberland et al., 2009).

This review focuses on mechanisms involved in non-transcriptional action of estrogens, in particular the metabolic and angiogenic responses mediated by estrogen-activated GPER signaling. The ability of estrogens in regulating glycolytic metabolism through microRNAs (miRNAs) in cancer will also be covered.

Protein Regulation by Post-Transcriptional and Epigenetic Mechanisms

Post-transcriptional regulation occurs in a variety of biologic processes (Corbett, 2018). However, a given process is often regulated through multiple mechanisms that afford fine-tuning and lead to cell responses within different time frames (Corbett, 2018). By way of example, one such process is the regulation of proteins involved in cellular energy production through glucose metabolism (Kim and Lee, 2012). Fast proliferating cells (e.g., tumor cells) must be able to tightly control and coordinate glucose metabolism and the cell cycle to guarantee sufficient ATP and anabolic substrates at distinct phases of the cell cycle (Huber et al., 2021). Protein production can be regulated at post-transcriptional levels by several mechanisms affecting mRNA and protein turnover (Mata et al., 2005). Regulation of mRNA stability of glycolytic proteins has been investigated in different cell types, mainly in cancer cells (Qi and Pekala, 1999; Wang et al., 2014). The 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) enzyme is a key player in glycolysis/

oxidative stress, and regulates metabolism and chemosensitivity in various cancer models (Mondal et al., 2019). The mRNA of all PFKFB3 isoforms is highly unstable. The *PFKFB3* gene generates many mRNA transcripts through alternative splicing (Kessler and Eschrich, 2001). A common feature of splicing variants is the presence of multiple copies of the AUUUA sequence in the 3'-untranslated region (UTR) (Bando et al., 2005). The AUUUA motif typifies the 3'-UTR region structures of several proto-oncogenes and proinflammatory cytokines and confers instability and enhanced translational activity to mRNAs (Shaw and Kamen, 1986). PFKFB3 is the first metabolic enzyme to be identified whose mRNA contains the AUUUA instability element (Chesney et al., 1999). Regulation of mRNA stability also plays a role in regulating glucose transporter 1 (GLUT1)/solute carrier family 2, facilitated glucose transporter member 1 gene expression. In terms of structure, *GLUT1* mRNA contains a 3'-UTR of 884 nucleotides and is considered to be A+U rich (Mueckler et al., 1985). Several laboratories have reported alterations in the stability of *GLUT1* transcript in various cell lines and tissues under conditions including glucose deprivation and hyperglycemia, hemangioblastoma as well as cytokine, hormone, and metabolite stimulation (Qi and Pekala, 1999).

In addition to the above-mentioned mechanisms that affect mRNA stability, cancer cells can adjust PFKFB3 activity according to their own metabolic demand both by varying *PFKFB3* gene expression and through post-translational protein modifications. For instance, S-glutathionylation results in decreased PFKFB3 activity (Seo and Lee, 2014), whereas phosphorylation enhances its activity and it is associated with increased glycolysis and cancer progression (Bando et al., 2005). Effects of phosphorylation on PFKFB3 activity have also been investigated in noncancer cells. For example, monocytes exposed to hypoxia rapidly stimulate glycolysis by activating PFKFB3 through protein phosphorylation on serine 461, located at the protein C-terminal domain (Marsin et al., 2002). Similar to PFKFB3, *GLUT1* undergoes post-translational modifications such as phosphorylation, resulting in increased protein activity; in particular, phosphorylation of *GLUT1* by protein kinase C on serine 226 enhances cell surface localization and glucose uptake in endothelial cells (Lee et al., 2015). Specific post-translational modifications including methylation and phosphorylation of specific residues promote proteasomal protein degradation. For example, in the case of reduced methylation, PFKFB3 activity is reduced as the enzyme undergoes polyubiquitination and is degraded by the proteasome (Yamamoto et al., 2014). Overall, the amount of glycolytic proteins such as PFKFB3 and *GLUT1* can be modulated via post-transcriptional mechanisms including protein translation and mRNA or protein stability. It is conceivable that the mechanisms involved in the regulation of key glycolytic promoters are not the same in healthy and tumor cells and/or vary depending on the stimulus.

Post-Transcriptional Regulation of Glycolytic Proteins and Angiogenesis by Estrogen

Endothelial cells are considered the body's largest endocrine organ, with a weight that is similar to the liver, covering a surface area of approximately 400 m² and counting approximately 1.2 billion cells in a healthy human (Anggård, 1990). Indeed, endothelial cells are metabolically highly active and rely primarily on glycolysis to produce energy in a short timeframe,

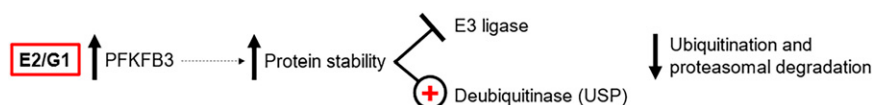


Fig. 1. Mechanisms of estrogen-dependent effects on PFKFB3 target gene stability. Estrogenic agents rapidly increase PFKFB3 levels by promoting protein stability, namely by decreasing PFKFB3 ubiquitination and proteasomal degradation. Treatment with 17 β -estradiol (E2) and the GPER agonist G1 enhances PFKFB3 protein stability via upregulation of USP19.

thus allowing rapid adaptation to micro-environmental changes (Eelen et al., 2015). In particular, GLUT1 and the glycolytic enzyme activator PFKFB3 play a relevant role in the control of endothelial function, including physiopathological angiogenesis. In fact, angiogenic signaling pathways converge into metabolism (Schoors et al., 2014; Draoui et al., 2017), and pathologic angiogenesis can be inhibited by blocking PFKFB3. Fructose-2,6 biphosphate, the product of PFKFB3, is the allosteric activator of the key glycolytic enzyme phosphofructokinase 1. In endothelial cells, which largely rely on glycolysis for ATP generation (Culic et al., 1997), PFKFB3 represents the most abundant PFKFB isoenzyme (De Bock et al., 2013). Because PFKFB3 plays a role in transcription and its product increases the expression and activity of cyclin-dependent-kinase-1 (Yalcin et al., 2009), PFKFB3 represents a novel pharmacological for targeting angiogenesis and tumor migration and cell growth. The glucose transporters GLUT1 and GLUT4, members of the solute carrier family 2, are responsible for taking up extracellular glucose into endothelial cells (Leung and Shi, 2022). It has also been shown that GLUT1 protein deficiency arrests angiogenesis, resulting in overt manifestations of brain dysfunction (Tang et al., 2017).

Estrogens are involved in metabolic regulation (Cignarella et al., 2010; Meyer et al., 2011). Specifically, whether estrogen modulates glycolysis by targeting the stability of glycolytic proteins, and in particular that of PFKFB3 and GLUT1, has been actively investigated. Both GLUT1 and PFKFB3 are finely regulated at both the transcriptional and post-transcriptional level by a variety of stimuli, including estrogens (Obach et al., 2004; Veschini et al., 2007). In particular, the biologically most relevant estrogen, 17 β -estradiol (E2), upregulates GLUT1 mRNA and protein levels, respectively, in different cell types including microvascular endothelial and cancer cells (Shi and Simpkins, 1997). In breast cancer cells, E2-induced alterations in glucose metabolism involve increased transcription of glycolytic proteins including PFKFB3 (Neeman and Degani, 1989; Imbert-Fernandez et al., 2014). Conversely, we reported that E2 rapidly increases PFKFB3 protein levels in a concentration-dependent manner without affecting *PFKFB3* mRNA levels (Trenti et al., 2017; Boscaro et al., 2020a), suggesting that nongenomic mechanisms drive estrogen-boosted glycolysis in the vascular endothelium. Thus, E2 promotes glycolytic metabolism in human endothelial cells, where PFKFB3 is a downstream effector of angiogenesis, highlighting that estrogens contribute to adaptable changes of endothelial glycolytic metabolism (Trenti et al., 2017).

The GPER can cross-talk and interact with classic membrane-associated and nuclear ERs (Barton, 2012; Prossnitz and Barton, 2023). Although the classic ERs have been acknowledged to function in mediating estrogen effects on glucose metabolism, metabolic and angiogenic actions of GPER have been elucidated in the vascular endothelium and in other cell types (Sharma and Prossnitz, 2021). As noted above, estrogens contribute to rapid adaptation of metabolic demand in

endothelial tissue by regulating glycolytic protein levels through post-translational mechanisms. GPER promotes glycolysis in endothelial cells and mediates the interface between estrogenic agents and PFKFB3 (Trenti et al., 2017; Boscaro et al., 2020a). In fact, treatment with E2 and the selective GPER agonist G1 increases PFKFB3 protein stability by enhancing ubiquitin-specific peptidase 19 (USP19) protein amount (Boscaro et al., 2020a), thus decreasing PFKFB3 ubiquitination and proteasomal degradation, resulting in enhanced angiogenesis (Fig. 1). This effect is already detectable within 60 minutes of exposure, as indicated by the rapid increase in PFKFB3 protein levels and the involvement of a membrane receptor. GPER silencing abolished the E2-mediated increase in USP19 levels, further supporting the role of GPER-dependent signaling pathways in the rapid post-transcriptional regulation of endothelial PFKFB3. It is tempting to speculate that estrogens could induce the phosphorylation of PFKFB3 in endothelial cells. Overall, these findings are consistent with the notion that estrogen rapidly promotes endothelial glycolysis through a variety of nongenomic mechanisms that may also account for at least some of the proangiogenic effect of estrogenic agents (reviewed in Cignarella et al., 2022).

Other Mechanisms of Nongenomic Protein Regulation by Estrogens

Some evidence shows that E2 affects the rate of protein translation, thereby modulating protein levels (Sudhagar et al., 2011). For instance, nanomolar concentrations of E2 increase protein S-nitrosylation in endothelial cells via ER α and nitric oxide synthase 3, also known as endothelial nitric oxide synthase, which attenuates the pro-inflammatory actions of angiotensin II (Chakrabarti et al., 2010). The selective ER modulator (SERM) raloxifene upregulates telomerase activity in HUVECs via increased phosphorylation through the PI3K/Akt cascade (Doshida et al., 2006). Through activation of the same pathway, E2 treatment increases programmed cell death ligand 1 (PD-L1) protein abundance in ER α -positive cancer cell lines as a result of increased mRNA stability (Yang et al., 2017). Finally, exposure to E2 or G1 rapidly stabilizes hypoxia-inducible factor 1- α protein with a peak effect at 15 minutes in endometrial tissue without affecting its mRNA levels (Zhang et al., 2017). This suggests that estrogen effects are mediated by a variety of mechanisms that do not involve the transcription of target genes.

Estrogen Signaling and Intracellular Protein Degradation via the Proteasomal Pathway

Regulation of protein levels due to changes in the degradation rate affords rapid adaptation to environmental changes; thus, key players in essential cellular pathways often undergo rapid ubiquitin-proteasome degradation (Stangl and Stangl,

2010). The ubiquitin/proteasome pathway is a possible mechanism for post-transcriptional regulation. The 8-kDa peptide ubiquitin is found in eukaryotes. Ubiquitin or a polyubiquitin chain is conjugated with the targeted protein to form polyubiquitinated protein complexes, which are delivered to and degraded by the 26S-proteasome (Miller and Gordon, 2005). The covalent bonding of ubiquitin occurs via the sequential action of four enzyme families: E1, E2, E3, and E4 (Micel et al., 2013). This process is finely tuned, and intracellular levels of ubiquitinated proteins at any given time depend on the balance between ubiquitination and deubiquitylation reactions in the ubiquitin-proteasome system (Ciechanover et al., 2000).

In noncancer cells, estrogens appear to modulate protein levels by targeting protein translation or proteasomal degradation rate, possibly via specific E3 ligases (Tschugguel et al., 2003). For instance, so-called conjugated estrogens, a mixture of hormones derived from pregnant horses' urine also containing equine estrogens, progestins and androgens (Barton et al., 2007), increase endoplasmic reticulum-associated degradation-associated E3 ubiquitin-protein ligase (HRD1) abundance without increasing *HRD1* mRNA levels in pancreatic beta-cells (Xu et al., 2018). Whether this also applies to human sex hormones, and which receptor pathways are activated by equine sex hormones is not clear. Through post-translational mechanisms involving stabilization of the E3 ubiquitin ligase HRD1, equine sex hormones also promote ubiquitination and proteasomal degradation of misfolded proteins via the endoplasmic reticulum-associated degradation system, thus preventing endoplasmic reticulum stress and beta-cell dysfunction in vitro and in vivo (Xu et al., 2018; Sachs et al., 2020). In skeletal muscle, E2 increases USP19 protein levels leading to reduced degradation of proteins that are involved in the repression of myogenesis (Ogawa et al., 2015). USP19 is a sex-specific potent down-regulator of muscle mass in young female mice (Ogawa et al., 2015). Systemic genetic deletion of ER α increases soleus muscle mass by repressing USP19 expression in female mice but not in male mice. By binding to ER α , E2 upregulates *Usp19* expression. USP19 removes ubiquitin from ubiquitinated target proteins, leading to skeletal muscle atrophy in young female mice (Ogawa et al., 2015). In humans, the impact of estrogen-containing menopausal hormonal therapy on muscle mass or strength is controversial, probably due to the use of a large number of different formulations (Ikeda et al., 2019). Of note, USP19 has also been identified as a potent inhibitor for tumor necrosis factor- α - and interleukin-1 β -induced activation of nuclear factor- κ B (Lei et al., 2019), suggesting a potential novel regulatory mechanism underlying the anti-inflammatory actions of estrogenic agents.

In cancer cells, estrogen promotes K-Ras stabilization by inhibiting its polyubiquitylation, thus contributing to endometrial cellular transformation and tumor growth (Koo et al., 2015). Estrogenic agents target both specific E3 ubiquitin ligases and USPs with different effects according to cell types. As an example, in endometrial cancer cells, estrogens promote the proteasomal degradation of the tumor suppressor p27, which is prevented by inhibitors of the SCF-Skp2/Cks E3 ligase (Pavlidis et al., 2013). Taken together, the complex interplay between estrogen signaling and proteasome regulation has been increasingly elucidated, and estrogenic agents appear to play a significant role in this process with consequences in health and disease (Xu et al., 2018).

Post-Transcriptional Modification of ER Expression and Function

Site-specific covalent post-translational modifications, including acetylation at lysines 266 and 268, increase both the DNA-binding and transcriptional activities of human ER α (Kim et al., 2006). Post-translational modifications impact on ER expression and stability, subcellular localization, and sensitivity to pharmacological agents. Discrepancy between ER gene expression and protein accumulation has been reported. For instance, short-term transdermal E2 treatment of 1 week, an increase in adipose tissue *ESR1* gene expression was observed in both early and late postmenopausal women, although ER α protein levels did not change (Park et al., 2017). This may be due to post-translational modification such as protein degradation or ubiquitination. In target tissues such as the uterine endothelium, E2 regulates expression of ER α by rapid degradation via a proteasome-mediated pathway (Tschugguel et al., 2003). ER palmitoylation, a protein modification determining its stability, trafficking to the plasma membrane and membrane signaling, is essential in mediating neuronal membrane-initiated E2 signaling (Meitzen et al., 2013). E2-bound ER α has been reported to be more susceptible to its degradation in the non-palmitoylated state (La Rosa et al., 2012). Mice expressing an ER α mutated for the palmitoylation site (C451A-ER α) are a model for membrane-induced steroid signaling loss-of-function (Adlanmerini et al., 2014). Of note, simvastatin treatment reduces ER α palmitoylation and increases ubiquitin-mediated ER α degradation in immortalized human leiomyoma cells (Afrin et al., 2021).

ER α signaling is regulated by other proteins and transcription factors. For example, resolvin D2, a member of the specialized pro-resolving lipid mediator family, indirectly modulates ER α signaling in ER-positive breast cancer cells transcription via activation of the PI3K/Akt signaling pathway (Al-Zaubai et al., 2014). Because of the interplay between growth factor and E2 signaling that regulates transcriptional effects (Marquez et al., 2001), resolvin D2 may activate signaling cascades leading to post-translational modifications of ER α or its co-activators or co-repressors (Al-Zaubai et al., 2014). FOXA1 is a transcription factor that enables ER binding to chromatin. In breast cancer cells, FOXA1 is responsible for most ER binding events in the genome and its upregulation is associated with enhancer reprogramming in endocrine resistance (Fu et al., 2016). Of note, FOXA1 is post-translationally modified in response to proinflammatory cytokines at two evolutionarily conserved amino acids (Franco et al., 2015). The post-translational modification of FOXA1 in response to external stimuli suggests a mechanism underlying FOXA1 regulation and function (Toska et al., 2017). In particular, estrogen-directed post-translational modification of FOXA1 may dictate binding site selection and drive ER α to non-canonical enhancers across the genome, thereby activating expression programs that underlie the tumorigenesis of breast cancer (Fu et al., 2019).

Pharmacological modification of ER stability may represent an interesting therapeutic approach. In particular, SERMs and selective ER antagonists and degraders (SERDs), such as fulvestrant, are approved for the treatment of ER-positive breast cancer. However, the emergence of mutations in the *ESR1* gene encoding ER α (*ESR1m*) (Scott et al., 2020) has been described as a major mechanism of resistance to endocrine-based therapy (O'Leary et al., 2018). Novel compounds are

likely to provide superior clinical benefit to existing endocrine therapies through improved target engagement and modulation in patients with hormone receptor-positive breast cancer. AZD9833 is an orally active, potent, next-generation SERD that has demonstrated antitumor activities in several ER-positive cell lines and patient-derived breast cancer xenograft models (Scott et al., 2020). It also holds promise to counteract or delay the emergence of endocrine resistance, which develops in patients treated with ER α -targeting drugs. A Phase I study of AZD9833 (SERENA-1) showed good oral bioavailability (Gangl et al., 2020) to support once daily oral administration. In addition, promising antitumor activities and an encouraging safety profile (Hamilton et al., 2020) were seen with AZD9833 monotherapy. In this context, although SERMs and SERDs inhibit cell survival and proliferation through the inhibition or degradation of ER α , respectively, at the same time they show agonistic effects toward GPER (Filardo et al., 2000; Thomas et al., 2005; Petrie et al., 2013). Sustained stimulation of GPER in women taking SERM/SERD therapies possibly represents one of the mechanisms contributing to resistance or even stimulation of certain recurring breast tumors, as well as the increased risk of endometrial hyperplasia and cancer (Pepermans and Prossnitz, 2019). This suggests that ER α inhibition in the absence of GPER activation might reduce anti-hormone therapy resistance with the future possibility of improved outcomes for women with breast cancer.

Accumulating evidence suggests that ER β shifts from a predominantly ligand-activated transcription factor (its major role during reproductive years) to a ligand-independent transcription factor after menopause with constitutive activity (Kim et al., 2018). The main molecular factors that facilitate the ligand-independent function of ER β in the aged brain are post-translational phosphorylation of the receptor (Pinceti et al., 2015), alternative RNA splicing, and coregulatory protein interactions. Therefore, understanding the basic molecular signaling pathways of E2 in the aging brain will help drive therapeutic advances and inform treatment strategies for postmenopausal women. In addition, GPER also drives constitutive, ligand-independent expression and activity on NADPH oxidase 1 (Meyer et al., 2016), an NADPH oxidase isoform that is key to many chronic diseases and is involved in the physiologic aging process (Zhu et al., 2015; Meyer et al., 2016; Tsai et al., 2016; Yoon et al., 2023).

Regulation of Protein Translation by miRNAs and the Role of Estrogen

The discovery of miRNAs, a class of small non-coding RNAs molecules comprising 22–25 nucleotides, has revealed the existence of a new level of gene expression regulation (Cech and Steitz, 2014). Post-transcriptional protein regulation includes several mechanisms affecting mRNA and protein turnover, which are not mutually exclusive (Maier et al., 2009). Among the post-transcriptional mechanisms involved in the regulation of protein levels, miRNAs act as negative post-transcriptional regulators of gene expression by targeting multiple mRNAs and inducing repression of translation and/or RNA degradation (Iorio et al., 2007; Chamorro-Jorganes et al., 2013; Oliveto et al., 2017). In particular, miRNAs mediate translational repression through direct binding to the 3'-UTR of target mRNA (Kong et al., 2008). In addition, miRNAs are emerging rapid

post-transcriptional regulators of protein abundance and function. The intracellular pool of functional miRNAs is controlled at multiple levels, including pre-miR maturation and miRNA degradation (Kir et al., 2018). Moreover, miRNAs are critical regulators of cell proliferation, differentiation, and cellular responses to stress, but their biogenesis is poorly understood. For example, transcription rates of miRNAs often do not correlate with the level of the corresponding mature miRNA, suggesting that post-transcriptional events determine the fate of miRNAs during these cellular processes (Ding et al., 2009). The miRNA pathway can be regulated through post-transcriptional modification. Specifically, the BCDIN3D RNA methyltransferase methylates specific miRNA precursors to inhibit their processing into mature miRNAs by Dicer (Xhemalce et al., 2012). Given that miRNAs are de-regulated in human diseases, obtaining a comprehensive mechanistic view of miRNA biogenesis could greatly expand potential therapeutic opportunities.

MiRNAs are recognized as rapid post-transcriptional regulators of protein abundance, and a single miRNA has the potential to simultaneously regulate multiple proteins (Fabian et al., 2010). Emerging evidence establishes miRNAs as novel players in the regulation of glycolytic metabolism in cancer cells (Subramaniam et al., 2019). Remarkably, the amount of PFKFB3 and GLUT1 is regulated by the action of specific miRNAs in several cancer cell types, and deregulation of miRNA expression has been attributed to estrogen-related cancers (Ge et al., 2015; He et al., 2019). There is strong evidence, at least in estrogen-sensitive cancer cells, for a role of miR-206 and miR-26b in the downregulation of PFKFB3 protein levels, which impacts on cell growth and migration (Du et al., 2015; Ge et al., 2015). Several miRNAs are up- or downregulated in ovarian cancer, suggesting that they play a role as a novel class of oncogenes or tumor-suppressor genes depending on the targets they regulate (Iorio et al., 2007). A further layer of complexity is due to the possible combination of the competing endogenous RNA phenomenon, whereby coding and noncoding RNA molecules targeted by the same miRNAs compete for binding and epigenetic silencing of miRNA by target proteins, as demonstrated using mathematical models (Huang et al., 2022). In noncancer cells, emerging evidence in post-ischemic tissues suggests a pro- or anti-angiogenic role of specific miRNAs in the regulation of metabolism and neovascularization depending on their targets (Azzouzi et al., 2015; Kir et al., 2018). Remarkably, mice lacking DICER, a key enzyme in miRNA processing maturation, display defective vessel formation and die (Singh et al., 2011). In addition, miRNAs affect angiogenesis at distant sites, being packaged into vesicles (exosomes) and secreted (Kir et al., 2018). Hence, plasma miRNAs could also represent disease biomarkers (Backes et al., 2016).

To date, over 200 estrogen-regulated miRNAs have been identified, and estrogen is known to suppress or stimulate miRNA expression and/or activity acting at multiple levels, including pre-miR maturation and miRNA degradation (Klinge, 2015). For example, E2 reduces endogenous miR-26b and miR-206 expression in the MCF-7 breast cancer cell line (Tan et al., 2014; Ge et al., 2015), which has been correlated with increased cell proliferation or migration. miR-206 negatively regulates estrogen-responsive genes in a complex interplay (Tan et al., 2014). Consistently, overexpression of these miRNAs reduces E2-dependent cell growth (Tan et al., 2014; Ge et al., 2015). In addition, E2 can regulate miRNA function by acting at the post-transcriptional level. There is evidence that disrupted ER α signaling in breast cancer is mediated by expression of

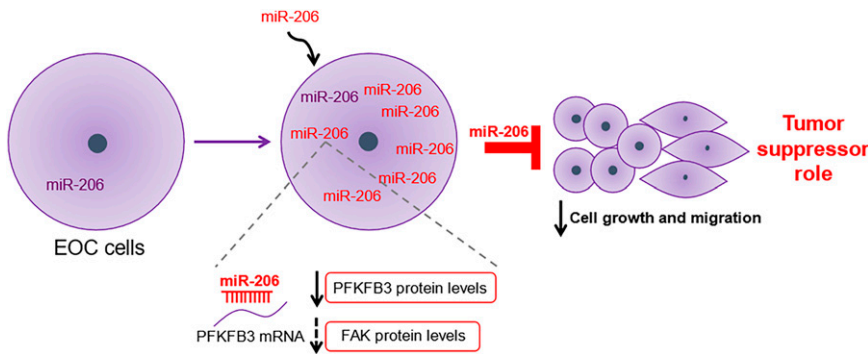


Fig. 2. Graphic depiction summarizing the tumor suppressor role of miR-206 in ovarian cancer cells. In cells expressing low levels of miR-206, exogenous administration of miR-206 induces antimigratory and antiproliferative effects through downregulation of the PFKFB3/FAK axis. EOC, epithelial ovarian cancer.

Argonaute 2 (AGO2), a protein that regulates translation and microRNA function (Conger et al., 2016). E2 has been shown to regulate AGO2 expression or phosphorylation by interacting with the EGF pathway (Adams et al., 2009). Notably, AGO2 can also be regulated by E3 ubiquitin ligases, which promote its degradation, thereby inhibiting miRNA activity (Klinge, 2015). An involvement of the “classical,” i.e. nuclear estrogen receptors (e.g., ER α) in miRNA regulation by estrogens has also been established (Klinge, 2015). Expression of the ER- α 36 variant in breast cancer cells is inversely correlated with *let-7* miRNAs, which also increase sensitivity to tamoxifen treatment in resistant cells (Zhao et al., 2011). Recently, GPER has been shown to mediate the inhibition of miR-338-3p expression induced by E2 in an ER-negative breast cancer cell line. Consistent with these observations, miR-338-3p mimics decrease E2-induced cancer cell proliferation, providing evidence for a novel role of GPER signaling in miRNA regulation (Vivacqua et al., 2018).

In ovarian cancer cells, E2 treatment increases PFKFB3 protein levels at early time points (3–6 hours) and for up to 24 hours. PFKFB3 upregulation occurs without changes in mRNA levels, in line with what is observed in human endothelial cells (Trenti et al., 2017; Boscaro et al., 2020a). Unexpectedly, the E2-dependent increase in PFKFB3 protein levels did not correlate with the growth response (Boscaro et al., 2022), suggesting additional levels of regulation of cell proliferation. Similarly, E2 treatment increases *c-myc* and *c-fos* mRNA levels in the absence of any proliferative response in SKOV3 cells (Hua et al., 1995). However, estrogen-induced growth has been associated with increased *c-fos* expression in several cancer cell lines (Liu et al., 2014), suggesting that the functional activation of ERs depends on the specific environment and cell type.

Based on the observation that E2 treatment increases PFKFB3 protein levels via non-genomic mechanisms (Trenti et al., 2017; Boscaro et al., 2020a), miRNAs may be involved in this regulation by targeting PFKFB3 3'-UTR. In fact, both miR-26b and miR-206 negatively regulate PFKFB3 expression in a luciferase assay (Boscaro et al., 2022). Transfection of SKOV3 cells with miR-26b and miR-206 significantly reduces PFKFB3 protein abundance, which is not reverted by E2 pretreatment (Boscaro et al., 2022). This is consistent with previous findings in breast cancer cells transfected with a miR-338-3p mimic, where treatment with E2 or G1 failed to upregulate *c-fos* mRNA and protein levels (Vivacqua et al., 2018). Conversely, miR-206 directly interacts with the 3'-UTR of *PFKFB3* mRNA in breast cancer cells lines, and E2 treatment was partially able to revert this miRNA effect on PFKFB3 levels (Ge et al., 2015). Overall, it appears that estrogenic agents can inhibit endogenous miRNAs' functions but cannot counteract the effect of long-term

exposure to exogenous miRNAs. Notably, both miR-206 and miR-26b are significantly downregulated in ovarian cancer with respect to normal tissue (Lin et al., 2015; Dai et al., 2018), consistent with the hypothesis that tissues permanently exposed to high estrogen concentrations express lower levels of specific miRNAs. miR-206 overexpression impairs proliferation and migration of estrogen-dependent cancer cells by modulating PFKFB3 amounts (Boscaro et al., 2022), which is functionally linked to protein tyrosine kinase 2 (FAK), a master regulator of cell migration (Boscaro et al., 2020b) (Fig. 2). miR-206 is downregulated in ER α -positive tissues and in MCF7 cells, and ER α agonists may further modulate this effect (Adams et al., 2007; Kondo et al., 2008). This is consistent with a direct effect of tumor-suppressor miR-206 on the negative regulation of estrogen-responsive genes, including ER α (Kondo et al., 2008; Chen et al., 2012).

Although estrogens can stimulate or suppress miRNA activity in different types of cancer cells, it is unclear whether this occurs in healthy tissues as well (Klinge, 2015; McCall et al., 2011). miR-7a is expressed in granulosa cells in the ovary, is involved in estrogen synthesis and regulates ovarian function (Li et al., 2022). Because miRNAs are rapid post-transcriptional regulators of protein abundance as well as emerging players in endothelial cell metabolism and function including angiogenesis, miRNAs may participate in the functional regulation of endothelial glycolytic programs, with a possible role for GPER in this process (Vidal-Gómez et al., 2018; Wade et al., 2019; Boscaro et al., 2020a). miR-mediated PFKFB3 downregulation may also be relevant in vascular ischemic disorders, where rapid metabolic and functional adaptation to environmental changes occurs (Li et al., 2019). Along the same lines, although the underlying basis for sexual dimorphism in autoimmune diseases is yet to be determined (Whitacre, 2001), the X chromosome is highly enriched for miRNAs, whose expression can be regulated by estrogens: dysregulated miRNA expression has been documented in some autoimmune disease conditions, including rheumatoid arthritis (Dai and Ahmed, 2011).

Epigenetic Regulation of Adipose Tissue by Estrogens

Reduced endogenous production of estrogens (e.g., after menopause) predisposes to or facilitates visceral adiposity (Papadakis et al., 2018). Adipose tissue morphology and biology in males and females in turn are differentially programmed by early life exposures, as has been suggested in some preclinical studies (Rodgers and Sferruzzi-Perri, 2021). Estrogen-mediated

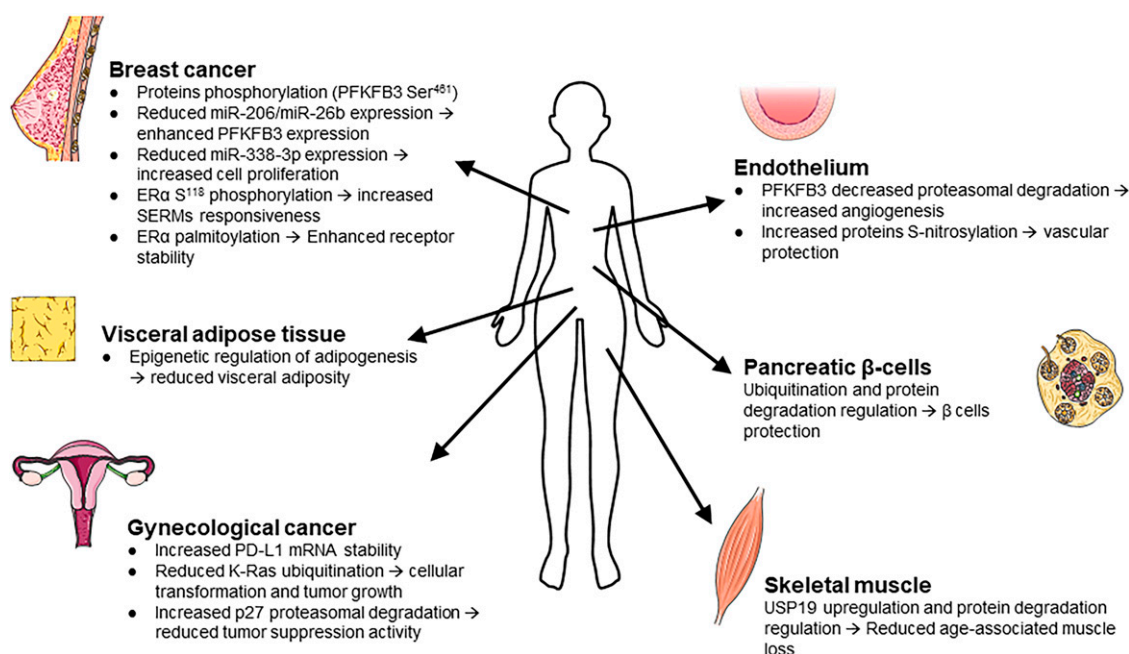


Fig. 3. Estrogen-regulated post-transcriptional mechanisms involved in different human diseases. Estrogens are involved in several regulatory mechanisms that do not require gene expression regulation. These include modulation of protein ubiquitination and degradation, phosphorylation, palmitoylation, nitrosylation, and miRNA expression, which have been associated with cancer, aging, and cardiovascular diseases. SERMs, selective ER modulators; PD-L1, programmed cell death 1 ligand 1.

epigenetic regulation of adipogenic genes via interaction with enzymes involved in DNA methylation and histone tail post-translational modifications are key mechanisms believed to mediate the interaction between the in-utero environment and clinical outcomes (Jorgensen et al., 2016). These events may mediate the transgenerational inheritance of adipose tissue regulation and obesity (Sun et al., 2019). Moreover, ER α and/or ER β are thought to be involved in the epigenetic regulation of adipogenesis (Bitirim et al., 2021). Histone modifications by ER α are described: for instance, ER α interacts with and promotes the activity of lysine methyltransferase 2B, which is also required for ER α -activated gene transcription (Shi et al., 2011).

Conclusion

In addition to their genomic effects, multiple estrogen-dependent effects and mechanisms do not involve transcription of target genes. The main pathways discussed in this article are summarized in Fig. 3. For instance, slowing down glycolytic protein turnover via estrogen receptors such as GPER allows estrogens to rapidly and finely tune functions in several cell types including cancer, endothelial and immune cells that rely on glycolysis to rapidly adapt to environmental cues (Li et al., 2019). These observations might have implications in estrogen's protective and prophylactic effects in chronic ischemic vascular or myocardial diseases as well as in heart failure, where rapid metabolic and functional adaptation to environmental changes is required (Barton and Meyer, 2020; Mauvais-Jarvis et al., 2020; DeFilippis et al., 2022; Shuaishuai et al., 2023). Identification of E2-targeted miRNAs, which in turn regulate glycolytic protein levels as well as cell growth and invasiveness, may pave the way to the development of miRNA-based treatments for blocking adverse hormone functions such as pathologic angiogenesis in E2-dependent

cancers. In addition, drugs affecting ER stability and turnover are possible strategies to overcome resistance to ER-dependent cancer endocrine therapy. Thus, post-translational and epigenetic regulation of estrogen signaling offers exciting new possibilities for the development of new therapeutics, particularly in the fields of oncology and cardiovascular diseases.

Data Availability

This article contains no datasets generated or analyzed during the current study.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Cignarella, Boscaro, Albiero, Bolego, Barton.

References

- Adams BD, Claffey KP, and White BA (2009) Argonaute-2 expression is regulated by epidermal growth factor receptor and mitogen-activated protein kinase signaling and correlates with a transformed phenotype in breast cancer cells. *Endocrinology* **150**:14–23.
- Adams BD, Furneaux H, and White BA (2007) The micro-ribonucleic acid (miRNA) miR-206 targets the human estrogen receptor- α (ER α) and represses ER α messenger RNA and protein expression in breast cancer cell lines. *Mol Endocrinol* **21**:1132–1147.
- Adlanmerini M, Solinhac R, Abot A, Fabre A, Raymond-Letron I, Guihot AL, Boudou F, Sautier L, Vessièrès E, Kim SH et al. (2014) Mutation of the palmitoylation site of estrogen receptor α in vivo reveals tissue-specific roles for membrane versus nuclear actions. *Proc Natl Acad Sci USA* **111**:E283–E290.
- Afrin S, El Sabeh M, Islam MS, Miyashita-Ishiwata M, Malik M, Catherino WH, Akimzhanov AM, Boehning D, Yang Q, Al-Hendy A et al. (2021) Simvastatin modulates estrogen signaling in uterine leiomyoma via regulating receptor palmitoylation, trafficking and degradation. *Pharmacol Res* **172**:105856.
- Alexander SP, Cidlowski JA, Kelly E, Mathie A, Peters JA, Veale EL, Armstrong JF, Faccenda E, Harding SD, Pawson AJ et al. (2021) The concise guide to pharmacology 2021/22: nuclear hormone receptors. *Br J Pharmacol* **178** (Suppl 1):S246–S263.
- Al-Zubair N, Johnstone CN, Leong MM, Li J, Rizzacasa M, and Stewart AG (2014) Resolvin D2 supports MCF-7 cell proliferation via activation of estrogen receptor. *J Pharmacol Exp Ther* **351**:172–180.
- Anbalagan M, Huderson B, Murphy L, and Rowan BG (2012) Post-translational modifications of nuclear receptors and human disease. *Nucl Recept Signal* **10**:e001.
- Anggård EE (1990) The endothelium—the body's largest endocrine gland? *J Endocrinol* **127**:371–375.

- Azzouzi HE, Leptidis S, Doevendans PA, and De Windt LJ (2015) HypoxamiRs: regulators of cardiac hypoxia and energy metabolism. *Trends Endocrinol Metab* **26**:502–508.
- Backes C, Meese E, and Keller A (2016) Specific miRNA disease biomarkers in blood, serum and plasma: challenges and prospects. *Mol Diagn Ther* **20**:509–518.
- Bando H, Atsumi T, Nishio T, Niwa H, Mishima S, Shimizu C, Yoshioka N, Bucala R, and Koike T (2005) Phosphorylation of the 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase/PPKFB3 family of glycolytic regulators in human cancer. *Clin Cancer Res* **11**:5784–5792.
- Barton M (2012) Position paper: the membrane estrogen receptor GPER—clues and questions. *Steroids* **77**:935–942.
- Barton M and Meyer MR (2020) Heart failure with preserved ejection fraction in women: new clues to causes and treatment. *JACC Basic Transl Sci* **5**:296–299.
- Barton M, Filardo EJ, Lolait SJ, Thomas P, Maggiolini M, and Prossnitz ER (2018) Twenty years of the G protein-coupled estrogen receptor GPER: historical and personal perspectives. *J Steroid Biochem Mol Biol* **176**:4–15.
- Barton M, Meyer MR, and Haas E (2007) Hormone replacement therapy and atherosclerosis in postmenopausal women: does aging limit therapeutic benefits? *Arterioscler Thromb Vasc Biol* **27**:1669–1672.
- Benayoun BA and Veitia RA (2009) A post-translational modification code for transcription factors: sorting through a sea of signals. *Trends Cell Biol* **19**:189–197.
- Bitirim CV, Ozer ZB, and Akcali KC (2021) Estrogen receptor alpha regulates the expression of adipogenic genes genetically and epigenetically in rat bone marrow-derived mesenchymal stem cells. *PeerJ* **9**:e12071.
- Bologa CG, Revankar CM, Young SM, Edwards BS, Arterburn JB, Kiselyov AS, Parker MA, Tkachenko SE, Savchuk NP, Sklar LA et al. (2006) Virtual and biomolecular screening converge on a selective agonist for GPR30. *Nat Chem Biol* **2**:207–212.
- Boscaro C, Baggio C, Carotti M, Sandonà D, Trevisi L, Cignarella A, and Bolego C (2022) Targeting of PFKFB3 with miR-206 but not miR-26b inhibits ovarian cancer cell proliferation and migration involving FAK downregulation. *FASEB J* **36**:e22140.
- Boscaro C, Carotti M, Albiero M, Trenti A, Fadini GP, Trevisi L, Sandonà D, Cignarella A, and Bolego C (2020a) Non-genomic mechanisms in the estrogen regulation of glycolytic protein levels in endothelial cells. *FASEB J* **34**:12768–12784.
- Boscaro C, Trenti A, Baggio C, Scapin C, Trevisi L, Cignarella A, and Bolego C (2020b) Sex differences in the pro-angiogenic response of human endothelial cells: focus on PFKFB3 and FAK activation. *Front Pharmacol* **11**:587221.
- Caroccia B, Seccia TM, Barton M, and Rossi GP (2016) Estrogen signaling in the adrenal cortex: implications for blood pressure sex differences. *Hypertension* **68**:840–848.
- Cech TR and Steitz JA (2014) The noncoding RNA revolution—trashing old rules to forge new ones. *Cell* **157**:77–94.
- Chakrabarti S, Lekontseva O, Peters A, and Davidge ST (2010) 17 β -Estradiol induces protein S-nitrosylation in the endothelium. *Cardiovasc Res* **85**:796–805.
- Chamorro-Jorganes A, Araldi E, and Suárez Y (2013) MicroRNAs as pharmacological targets in endothelial cell function and dysfunction. *Pharmacol Res* **75**:15–27.
- Chen X, Yan Q, Li S, Zhou L, Yang H, Yang Y, Liu X, and Wan X (2012) Expression of the tumor suppressor miR-206 is associated with cellular proliferative inhibition and impairs invasion in ER α -positive endometrioid adenocarcinoma. *Cancer Lett* **314**:41–53.
- Chesney J, Mitchell R, Benigni F, Bacher M, Spiegel L, Al-Abed Y, Han JH, Metz C, and Bucala R (1999) An inducible gene product for 6-phosphofructo-2-kinase with an AU-rich instability element: role in tumor cell glycolysis and the Warburg effect. *Proc Natl Acad Sci USA* **96**:3047–3052.
- Ciechanover A, Orian A, and Schwartz AL (2000) The ubiquitin-mediated proteolytic pathway: mode of action and clinical implications. *J Cell Biochem Suppl* **34**:40–51.
- Cignarella A, Fadini GP, Bolego C, Trevisi L, Boscaro C, Sanga V, Seccia TM, Rosato A, Rossi GP, and Barton M (2022) Clinical efficacy and safety of angiogenesis inhibitors: sex differences and current challenges. *Cardiovasc Res* **118**:988–1003.
- Cignarella A, Kratz M, and Bolego C (2010) Emerging role of estrogen in the control of cardiometabolic disease. *Trends Pharmacol Sci* **31**:183–189.
- Conger AK, Martin EC, Yan TJ, Rhodes LV, Hoang VT, La J, Anbalagan M, Burks HE, Rowan BG, Nephew KP et al. (2016) Argonaute 2 expression correlates with a luminal B breast cancer subtype and induces estrogen receptor alpha isoform variation. *Noncoding RNA* **2**:8.
- Corbett AH (2018) Post-transcriptional regulation of gene expression and human disease. *Curr Opin Cell Biol* **52**:96–104.
- Culic O, Gruwel ML, and Schrader J (1997) Energy turnover of vascular endothelial cells. *Am J Physiol* **273**:C205–C213.
- Dai C, Xie Y, Zhuang X, and Yuan Z (2018) MiR-206 inhibits epithelial ovarian cancer cells growth and invasion via blocking c-Met/AKT/mTOR signaling pathway. *Biomol Pharmacother* **104**:763–770.
- Dai R and Ahmed SA (2011) MicroRNA, a new paradigm for understanding immunoregulation, inflammation, and autoimmune diseases. *Transl Res* **157**:163–179.
- De Bock K, Georgiadou M, Schoors S, Kuchnio A, Wong BW, Cantelmo AR, Quaegebeur A, Ghesquière B, Cauwenberghs S, Eelen G et al. (2013) Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell* **154**:651–663.
- DeFilippis EM, Beale A, Martyn T, Agarwal A, Elkayam U, Lam CSP, and Hsieh E (2022) Heart failure subtypes and cardiomyopathies in women. *Circ Res* **130**:436–454.
- Deroo BJ and Korach KS (2006) Estrogen receptors and human disease. *J Clin Invest* **116**:561–570.
- Ding XC, Weiler J, and Grosshans H (2009) Regulating the regulators: mechanisms controlling the maturation of microRNAs. *Trends Biotechnol* **27**:27–36.
- Doshida M, Ohmichi M, Tsutsumi S, Kawagoe J, Takahashi T, Du B, Mori-Abe A, Ohta T, Saitoh-Sekiguchi M, Takahashi K et al. (2006) Raloxifene increases proliferation and up-regulates telomerase activity in human umbilical vein endothelial cells. *J Biol Chem* **281**:24270–24278.
- Draoui N, de Zeeuw P, and Carmeliet P (2017) Angiogenesis revisited from a metabolic perspective: role and therapeutic implications of endothelial cell metabolism. *Open Biol* **7**:170219.
- Drazic A, Myklebust LM, Ree R, and Arnesen T (2016) The world of protein acetylation. *Biochim Biophys Acta* **1864**:1372–1401.
- Du JY, Wang LF, Wang Q, and Yu LD (2015) miR-26b inhibits proliferation, migration, invasion and apoptosis induction via the downregulation of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 driven glycolysis in osteosarcoma cells. *Oncol Rep* **33**:1890–1898.
- Eelen G, de Zeeuw P, Simons M, and Carmeliet P (2015) Endothelial cell metabolism in normal and diseased vasculature. *Circ Res* **116**:1231–1244.
- Fabian MR, Sonenberg N, and Filipowicz W (2010) Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem* **79**:351–379.
- Filardo EJ, Quinn JA, Bland KI, and Frackelton Jr AR (2000) Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. *Mol Endocrinol* **14**:1649–1660.
- Franco HL, Nagari A, and Kraus WL (2015) TNF α signaling exposes latent estrogen receptor binding sites to alter the breast cancer cell transcriptome. *Mol Cell* **58**:21–34.
- Fu X, Jeselsohn R, Pereira R, Hollingsworth EF, Creighton CJ, Li F, Shea M, Nardone A, De Angelis C, Heiser LM et al. (2016) FOXA1 overexpression mediates endocrine resistance by altering the ER transcriptome and IL-8 expression in ER-positive breast cancer. *Proc Natl Acad Sci USA* **113**:E6600–E6609.
- Fu X, Pereira R, De Angelis C, Veeraraghavan J, Nanda S, Qin L, Cataldo ML, Sethunath V, Mehravaran S, Gutierrez C et al. (2019) FOXA1 upregulation promotes enhancer and transcriptional reprogramming in endocrine-resistant breast cancer. *Proc Natl Acad Sci USA* **116**:26823–26834.
- Gangl ET, Markandu R, Sharma P, Sykes A, Pop-Damkov P, Gutierrez PM et al (2020) Prediclinical pharmacokinetic and metabolic characterization of the next generation oral SERD AZD9833. (Abstract) *Cancer Res* **80** (Suppl 16).
- Ge X, Lyu P, Cao Z, Li J, Guo G, Xia W, and Gu Y (2015) Overexpression of miR-206 suppresses glycolysis, proliferation and migration in breast cancer cells via PFKFB3 targeting. *Biochem Biophys Res Commun* **463**:1115–1121.
- Haberland M, Montgomery RL, and Olson EN (2009) The many roles of histone deacetylases in development and physiology: implications for disease and therapy. *Nat Rev Genet* **10**:32–42.
- Hall JM and McDonnell DP (2005) Coregulators in nuclear estrogen receptor action: from concept to therapeutic targeting. *Mol Interv* **5**:343–357.
- Hamilton EP, Oliveira M, Banerji U, Hernando C, Garcia-Corbacho J, Armstrong AC, Ciruelos E, Patel MR, Incorvati J, Twelves C, et al. (2020) A phase I dose escalation and expansion study of the next generation oral SERD AZD9833 in women with ER-positive, HER2-negative advanced breast cancer. *J Clin Oncol* **38**:1024.
- He Y, Deng F, Zhao S, Zhong S, Zhao J, Wang D, Chen X, Zhang J, Hou J, Zhang W et al. (2019) Analysis of miRNA-mRNA network reveals miR-140-5p as a suppressor of breast cancer glycolysis via targeting GLUT1. *Epigenomics* **11**:1021–1036.
- Hewitt SC and Korach KS (2018) Estrogen receptors: new directions in the new millennium. *Endocr Rev* **39**:664–675.
- Hu W and Coller J (2012) What comes first: translational repression or mRNA degradation? The deepening mystery of microRNA function. *Cell Res* **22**:1322–1324.
- Hua W, Christianson T, Rougeot C, Rochefort H, and Clinton GM (1995) SKOV3 ovarian carcinoma cells have functional estrogen receptor but are growth-resistant to estrogen and antiestrogens. *J Steroid Biochem Mol Biol* **55**:279–289.
- Huang TW, Cheng FHC, Yan CS, Chuang YM, Cho CH, Lai HC, Shieh SF, Chan MWY, and Tsai JC (2022) Interplay between ceRNA and epigenetic control of microRNA: modelling approaches with application to the role of estrogen in ovarian cancer. *Int J Mol Sci* **23**:2277.
- Huber K, Mestres-Arenas A, Fajas L, and Leal-Esteban LC (2021) The multifaceted role of cell cycle regulators in the coordination of growth and metabolism. *FEBS J* **288**:3813–3833.
- Ikeda K, Horie-Inoue K, and Inoue S (2019) Functions of estrogen and estrogen receptor signaling on skeletal muscle. *J Steroid Biochem Mol Biol* **191**:105375.
- Imbert-Fernandez Y, Clem BF, O'Neal J, Kerr DA, Spaulding R, Lanceta L, Clem AL, Telang S, and Chesney J (2014) Estradiol stimulates glucose metabolism via 6-phosphofructo-2-kinase (PFKFB3). *J Biol Chem* **289**:9440–9448.
- Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, Taccioli C, Volinia S, Liu CG, Alder H et al. (2007) MicroRNA signatures in human ovarian cancer. *Cancer Res* **67**:8699–8707.
- Jorgensen EM, Alderman 3rd MH, and Taylor HS (2016) Preferential epigenetic programming of estrogen response after in utero xenoestrogen (bisphenol-A) exposure. *FASEB J* **30**:3194–3201.
- Kessler R and Eschrich K (2001) Splice isoforms of ubiquitous 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase in human brain. *Brain Res Mol Brain Res* **87**:190–195.
- Kim CK, Torcaso A, Asimes A, Chung WCJ, and Pak TR (2018) Structural and functional characteristics of oestrogen receptor β splice variants: Implications for the ageing brain. *J Neuroendocrinol* **30**:10.1111/jne.12488.
- Kim MY, Woo EM, Chong YT, Homenko DR, and Kraus WL (2006) Acetylation of estrogen receptor alpha by p300 at lysines 266 and 268 enhances the deoxyribonucleic acid binding and transactivation activities of the receptor. *Mol Endocrinol* **20**:1479–1493.
- Kim W and Lee EK (2012) Post-transcriptional regulation in metabolic diseases. *RNA Biol* **9**:772–780.
- Kir D, Schnettler E, Modi S, and Ramakrishnan S (2018) Regulation of angiogenesis by microRNAs in cardiovascular diseases. *Angiogenesis* **21**:699–710.
- Klinge CM (2015) miRNAs regulated by estrogens, tamoxifen, and endocrine disruptors and their downstream gene targets. *Mol Cell Endocrinol* **418**:273–297.
- Kondo N, Toyama T, Sugiyama H, Fujii Y, and Yamashita H (2008) miR-206 Expression is down-regulated in estrogen receptor α -positive human breast cancer. *Cancer Res* **68**:5004–5008.

- Kong YW, Cannell IG, de Moor CH, Hill K, Garside PG, Hamilton TL, Meijer HA, Dobbryn HC, Stoneley M, Spriggs KA et al. (2008) The mechanism of micro-RNA-mediated translation repression is determined by the promoter of the target gene. *Proc Natl Acad Sci USA* **105**:8866–8871.
- Koo K-H, Jeong W-J, Cho Y-H, Park J-C, Min S, and Choi K-Y (2015) K-Ras stabilization by estrogen via PKC δ is involved in endometrial tumorigenesis. *Oncotarget* **6**:21328–21340.
- La Rosa P, Pesiri V, Leclercq G, Marino M, and Acconcia F (2012) Palmitoylation regulates 17 β -estradiol-induced estrogen receptor- α degradation and transcriptional activity. *Mol Endocrinol* **26**:762–774.
- Lee EE, Ma J, Sacharidou A, Mi W, Salato VK, Nguyen N, Jiang Y, Pascual JM, North PE, Shaul PW et al. (2015) A Protein Kinase C phosphorylation motif in GLUT1 affects glucose transport and is mutated in GLUT1 deficiency syndrome. *Mol Cell* **58**:845–853.
- Lei CQ, Wu X, Zhong X, Jiang L, Zhong B, and Shu HB (2019) USP19 Inhibits TNF- α and IL-1 β -Triggered NF- κ B Activation by Deubiquitinating TAK1. *J Immunol* **203**:259–268.
- Leung SWS and Shi Y (2022) The glycolytic process in endothelial cells and its implications. *Acta Pharmacol Sin* **43**:251–259.
- Levin ER and Hammes SR (2016) Nuclear receptors outside the nucleus: extranuclear signalling by steroid receptors. *Nat Rev Mol Cell Biol* **17**:783–797.
- Li L, Zhang J, Lu C, Wang B, Guo J, Zhang H, and Cui S (2022) MicroRNA-7a2 contributes to estrogen synthesis and is modulated by FSH via the JNK signaling pathway in ovarian granulosa cells. *Int J Mol Sci* **23**:8565.
- Li X, Sun X, and Carmeliet P (2019) Hallmarks of endothelial cell metabolism in health and disease. *Cell Metab* **30**:414–433.
- Lin J, Zhang L, Huang H, Huang Y, Huang L, Wang J, Huang S, He L, Zhou Y, Jia W et al. (2015) MiR-26b/KPNA2 axis inhibits epithelial ovarian carcinoma proliferation and metastasis through downregulating OCT4. *Oncotarget* **6**:23793–23806.
- Liu H, Yan Y, Wen H, Jiang X, Cao X, Zhang G, and Liu G (2014) A novel estrogen receptor GPER mediates proliferation induced by 17 β -estradiol and selective GPER agonist G-1 in estrogen receptor α (ER α)-negative ovarian cancer cells. *Cell Biol Int* **38**:631–638.
- Maier T, Güell M, and Serrano L (2009) Correlation of mRNA and protein in complex biological samples. *FEBS Lett* **583**:3966–3973.
- Márquez DC, Lee J, Lin T, and Pietras RJ (2001) Epidermal growth factor receptor and tyrosine phosphorylation of estrogen receptor. *Endocrine* **16**:73–81.
- Marsin AS, Bouzin C, Bertrand L, and Hue L (2002) The stimulation of glycolysis by hypoxia in activated monocytes is mediated by AMP-activated protein kinase and inducible 6-phosphofructo-2-kinase. *J Biol Chem* **277**:30778–30783.
- Mata J, Marguerat S, and Bähler J (2005) Post-transcriptional control of gene expression: a genome-wide perspective. *Trends Biochem Sci* **30**:506–514.
- Matthews J and Gustafsson JÅ (2003) Estrogen signaling: a subtle balance between ER α and ER β . *Mol Interv* **3**:281–292.
- Mauvais-Jarvis F, Bairey Merz N, Barnes PJ, Brinton RD, Carrero JJ, DeMeo DL, De Vries GJ, Epperson CN, Govindan R, Klein SL et al. (2020) Sex and gender: modifiers of health, disease, and medicine. *Lancet* **396**:565–582.
- Mauvais-Jarvis F, Lange CA, and Levin ER (2022) Membrane-initiated estrogen, androgen, and progesterone receptor signaling in health and disease. *Endocr Rev* **43**:720–742.
- McCall MN, Kent OA, Yu J, Fox-Talbot K, Zaiman AL, and Halushka MK (2011) MicroRNA profiling of diverse endothelial cell types. *BMC Med Genomics* **4**:78.
- Meitzen J, Luoma JJ, Boulware MI, Hedges VL, Peterson BM, Tuomela K, Britson KA, and Mermelstein PG (2013) Palmitoylation of estrogen receptors is essential for neuronal membrane signaling. *Endocrinology* **154**:4293–4304.
- Meyer MR, Clegg DJ, Prossnitz ER, and Barton M (2011) Obesity, insulin resistance and diabetes: sex differences and role of oestrogen receptors. *Acta Physiol (Oxf)* **203**:259–269.
- Meyer MR, Fredette NC, Daniel C, Sharma G, Amann K, Arterburn JB, Barton M, and Prossnitz ER (2016) Obligatory role for GPER in cardiovascular aging and disease. *Sci Signal* **9**:ra105.
- Micel LN, Tentler JJ, Smith PG, and Eckhardt GS (2013) Role of ubiquitin ligases and the proteasome in oncogenesis: novel targets for anticancer therapies. *J Clin Oncol* **31**:1231–1238.
- Miller J and Gordon C (2005) The regulation of proteasome degradation by multi-ubiquitin chain binding proteins. *FEBS Lett* **579**:3224–3230.
- Mondal S, Roy D, Sarkar Bhattacharya S, Jin L, Jung D, Zhang S, Kalogera E, Staub J, Wang Y, Xuyang W et al. (2019) Therapeutic targeting of PFKFB3 with a novel glycolytic inhibitor PFK158 promotes lipophagy and chemosensitivity in gynecologic cancers. *Int J Cancer* **144**:178–189.
- Mueckler M, Caruso C, Baldwin SA, Panico M, Blench I, Morris HR, Allard WJ, Lienhard GE, and Lodish HF (1985) Sequence and structure of a human glucose transporter. *Science* **229**:941–945.
- Neeman M and Degani H (1989) Early estrogen-induced metabolic changes and their inhibition by actinomycin D and cycloheximide in human breast cancer cells: 31 P and 13 C NMR studies. *Proc Natl Acad Sci USA* **86**:5585–5589.
- O'Leary B, Cutts RJ, Liu Y, Hrebien S, Huang X, Fenwick K, André F, Loibl S, Loi S, Garcia-Murillas I et al. (2018) The genetic landscape and clonal evolution of breast cancer resistance to palbociclib plus fulvestrant in the PALOMA-3 trial. *Cancer Discov* **8**:1390–1403.
- Obach M, Navarro-Sabaté A, Caro J, Kong X, Duran J, Gómez M, Perales JC, Ventura F, Rosa JL, and Bartrons R (2004) 6-Phosphofructo-2-kinase (*pfkfb3*) gene promoter contains hypoxia-inducible factor-1 binding sites necessary for transactivation in response to hypoxia. *J Biol Chem* **279**:53562–53570.
- OGawa M, Kitakaze T, Harada N, and Yamaji R (2015) Female-specific regulation of skeletal muscle mass by USP19 in young mice. *J Endocrinol* **225**:135–145.
- Oliveto S, Mancino M, Manfrini N, and Biffo S (2017) Role of microRNAs in translation regulation and cancer. *World J Biol Chem* **8**:45–56.
- Papadakis GE, Hans D, Gonzalez Rodriguez E, Vollenweider P, Waeber G, Marques-Vidal P, and Lamy O (2018) Menopausal hormone therapy is associated with reduced total and visceral adiposity: the OsteoLaus cohort. *J Clin Endocrinol Metab* **103**:1948–1957.
- Park YM, Pereira RI, Erickson CB, Swibas TA, Cox-York KA, and Van Pelt RE (2017) Estradiol-mediated improvements in adipose tissue insulin sensitivity are related to the balance of adipose tissue estrogen receptor α and β in postmenopausal women. *PLoS One* **12**:e0176446.
- Pavlidis SC, Huang KT, Reid DA, Wu L, Blank SV, Mittal K, Guo L, Rothenberg E, Rueda B, Cardozo T et al. (2013) Inhibitors of SCF-Skp2/Cks1 E3 ligase block estrogen-induced growth stimulation and degradation of nuclear p27kip1: therapeutic potential for endometrial cancer. *Endocrinology* **154**:4030–4045.
- Pepermans RA and Prossnitz ER (2019) ER α -targeted endocrine therapy, resistance and the role of GPER. *Steroids* **152**:108493.
- Petrie WK, Dennis MK, Hu C, Dai D, Arterburn JB, Smith HO, Hathaway HJ, and Prossnitz ER (2013) G protein-coupled estrogen receptor-selective ligands modulate endometrial tumor growth. *Obstet Gynecol Int* **2013**:472720.
- Pinceti E, Shults CL, Rao YS, Mott NN, and Pak TR (2015) Phosphorylation alters estrogen receptor β -mediated transcription in neurones. *J Neuroendocrinol* **27**:861–871.
- Prossnitz ER and Barton M (2023) The G protein-coupled estrogen receptor GPER in health and disease: an update. *Nat Rev Endocrinol* **19**:407–424.
- Qi C and Pekala PH (1999) The influence of mRNA stability on glucose transporter (GLUT1) gene expression. *Biochem Biophys Res Commun* **263**:265–269.
- Rodgers A and Sferruzzi-Perri AN (2021) Developmental programming of offspring adipose tissue biology and obesity risk. *Int J Obes* **45**:1170–1192.
- Sachs S, Bastidas-Ponce A, Tritschler S, Bakhti M, Böttcher A, Sánchez-Garrido MA, Tarquis-Medina M, Kleinert M, Fischer K, Jall S et al. (2020) Targeted pharmacological therapy restores β -cell function for diabetes remission. *Nat Metab* **2**:192–209.
- Schaefer B, Sun W, Li YS, Fang L, and Chen W (2018) The evolution of posttranscriptional regulation. *Wiley Interdiscip Rev RNA* **9**:e1485.
- Schoors S, De Bock K, Cantelmo AR, Georgiadou M, Ghesquière B, Cauwenberghs S, Kuchnio A, Wong BW, Quaebeur A, Goveia J et al. (2014) Partial and transient reduction of glycolysis by PFKFB3 blockade reduces pathological angiogenesis. *Cell Metab* **19**:37–48.
- Scott JS, Moss TA, Balazs A, Barlaam B, Breed J, Carbajo RJ, Chiarparin E, Davey PRJ, Delpuech O, Fawell S et al. (2020) Discovery of AZD9833, a potent and orally bioavailable selective estrogen receptor degrader and antagonist. *J Med Chem* **63**:14530–14559.
- Seo M and Lee YH (2014) PFKFB3 regulates oxidative stress homeostasis via its S-glutathionylation in cancer. *J Mol Biol* **426**:830–842.
- Sharma G and Prossnitz ER (2021) Targeting the G protein-coupled estrogen receptor (GPER) in obesity and diabetes. *Endocr Metab* **32**:100080.
- Shaw G and Kamen R (1986) A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell* **46**:659–667.
- Shi J and Simpkins JW (1997) 17 beta-Estradiol modulation of glucose transporter 1 expression in blood-brain barrier. *Am J Physiol* **272**:E1016–E1022.
- Shi L, Sun L, Li Q, Liang J, Yu W, Yi X, Yang X, Li Y, Han X, Zhang Y et al. (2011) Histone demethylase JMD12B coordinates H3K4/H3K9 methylation and promotes hormonally responsive breast carcinogenesis. *Proc Natl Acad Sci USA* **108**:7541–7546.
- Shuaishuai D, Jingyi L, Zhiqiang Z, and Guanwei F (2023) Sex differences and related estrogenic effects in heart failure with preserved ejection fraction. *Heart Fail Rev* **28**:937–948.
- Singh MK, Lu MM, Massera D, and Epstein JA (2011) MicroRNA-processing enzyme Dicer is required in epicardium for coronary vasculature development. *J Biol Chem* **286**:41036–41045.
- Stangl K and Stangl V (2010) The ubiquitin-proteasome pathway and endothelial (dys)function. *Cardiovasc Res* **85**:281–290.
- Subramaniam S, Jeet V, Clements JA, Gunter JH, and Batra J (2019) Emergence of microRNAs as key players in cancer cell metabolism. *Clin Chem* **65**:1090–1101.
- Sudhagar S, Sathya S, and Lakshmi BS (2011) Rapid non-genomic signalling by 17 β -estradiol through c-Src involves mTOR-dependent expression of HIF-1 α in breast cancer cells. *Br J Cancer* **105**:953–960.
- Sun W, von Meyenn F, Peleg-Raibstein D, and Wolfrum C (2019) Environmental and nutritional effects regulating adipose tissue function and metabolism across generations. *Adv Sci (Weinh)* **6**:1900275.
- Tan S, Ding K, Li R, Zhang W, Li G, Kong X, Qian P, Lobie PE, and Zhu T (2014) Identification of miR-26 as a key mediator of estrogen stimulated cell proliferation by targeting CHD1, GREB1 and KPNA2. *Breast Cancer Res* **16**:R40.
- Tang M, Gao G, Rueda CB, Yu H, Thibodeaux DN, Awano T, Engelstad KM, Sanchez-Quintero MJ, Yang H, Li F et al. (2017) Brain microvasculature defects and Glut1 deficiency syndrome averted by early repletion of the glucose transporter-1 protein. *Nat Commun* **8**:14152.
- Thomas P, Pang Y, Filardo EJ, and Dong J (2005) Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology* **146**:624–632.
- Toska E, Osmanbeyoglu HU, Castel P, Chan C, Hendrickson RC, Elkabets M, Dickler MN, Scaltriti M, Leslie CS, Armstrong SA et al. (2017) PI3K pathway regulates ER-dependent transcription in breast cancer through the epigenetic regulator KMT2D. *Science* **355**:1324–1330.
- Trenti A, Tedesco S, Boscaro C, Ferri N, Cignarella A, Trevisi L, and Bolego C (2017) The glycolytic enzyme PFKFB3 is involved in estrogen-mediated angiogenesis via GPER1. *J Pharmacol Exp Ther* **361**:398–407.
- Tsai IC, Pan ZC, Cheng HP, Liu CH, Lin BT, and Jiang MJ (2016) Reactive oxygen species derived from NADPH oxidase 1 and mitochondria mediate angiotensin II-induced smooth muscle cell senescence. *J Mol Cell Cardiol* **98**:18–27.
- Tschugguel W, Dietrich W, Zhegu Z, Stonek F, Kolbus A, and Huber JC (2003) Differential regulation of proteasome-dependent estrogen receptor α and β turnover in cultured human uterine artery endothelial cells. *J Clin Endocrinol Metab* **88**:2281–2287.
- Veschini L, Belloni D, Foglieni C, Cangi MG, Ferrarini M, Caligaris-Cappio F, and Ferrero E (2007) Hypoxia-inducible transcription factor-1 alpha determines

- sensitivity of endothelial cells to the proteasome inhibitor bortezomib. *Blood* **109**: 2565–2570.
- Vidal-Gómez X, Pérez-Cremades D, Mompeón A, Dantas AP, Novella S, and Herme-negildo C (2018) MicroRNA as crucial regulators of gene expression in estradiol-treated human endothelial cells. *Cell Physiol Biochem* **45**:1878–1892.
- Vivacqua A, Sebastiani A, Miglietta AM, Rigracciolo DC, Cirillo F, Galli GR, Talia M, Santolla MF, Lappano R, Giordano F et al. (2018) miR-338-3p Is regulated by estrogens through GPER in breast cancer cells and cancer-associated fibroblasts (CAFs). *Cells* **7**:203.
- Wade SM, Ohnesorge N, McLoughlin H, Biniecka M, Carter SP, Trenkman M, Cunningham CC, McGarry T, Canavan M, Kennedy BN et al. (2019) Dysregulated miR-125a promotes angiogenesis through enhanced glycolysis. *EBioMedicine* **47**:402–413.
- Wang L, Xiong H, Wu F, Zhang Y, Wang J, Zhao L, Guo X, Chang LJ, Zhang Y, You MJ et al. (2014) Hexokinase 2-mediated Warburg effect is required for PTEN- and p53-deficiency-driven prostate cancer growth. *Cell Rep* **8**:1461–1474.
- Whitacre CC (2001) Sex differences in autoimmune disease. *Nat Immunol* **2**:777–780.
- Xhemalce B, Robson SC, and Kouzarides T (2012) Human RNA methyltransferase BCDIN3D regulates microRNA processing. *Cell* **151**:278–288.
- Xu B, Allard C, Alvarez-Mercado AI, Fuselier T, Kim JH, Coons LA, Hewitt SC, Urano F, Korach KS, Levin ER et al. (2018) Estrogens promote misfolded proinsulin degradation to protect insulin production and delay diabetes. *Cell Rep* **24**:181–196.
- Yalcin A, Clem BF, Simmons A, Lane A, Nelson K, Clem AL, Brock E, Siow D, Wattenberg B, Telang S et al. (2009) Nuclear targeting of 6-phosphofructo-2-kinase (PFKFB3) increases proliferation via cyclin-dependent kinases. *J Biol Chem* **284**: 24223–24232.
- Yamamoto T, Takano N, Ishiwata K, Ohmura M, Nagahata Y, Matsuura T, Kamata A, Sakamoto K, Nakanishi T, Kubo A et al. (2014) Reduced methylation of PFKFB3 in cancer cells shunts glucose towards the pentose phosphate pathway. *Nat Commun* **5**:3480.
- Yang L, Huang F, Mei J, Wang X, Zhang Q, Wang H, Xi M, and You Z (2017) Post-transcriptional control of PD-L1 expression by 17 β -estradiol via PI3K/Akt signaling pathway in ER α -positive cancer cell lines. *Int J Gynecol Cancer* **27**:196–205.
- Yoon SG, Ghee JY, Yoo JA, Park BY, Cha JJ, Kang YS, Han SY, Min HS, Lee JE, Han JY et al. (2023) Role of NADPH oxidases in renal aging. *Gerontology* DOI: 10.1159/000529392.
- Zhang L, Xiong W, Li N, Liu H, He H, Du Y, Zhang Z, and Liu Y (2017) Estrogen stabilizes hypoxia-inducible factor 1 α through G protein-coupled estrogen receptor 1 in eutopic endometrium of endometriosis. *Fertil Steril* **107**:439–447.
- Zhao Y, Deng C, Lu W, Xiao J, Ma D, Guo M, Recker RR, Gatalica Z, Wang Z, and Xiao GG (2011) let-7 microRNAs induce tamoxifen sensitivity by downregulation of estrogen receptor α signaling in breast cancer. *Mol Med* **17**:1233–1241.
- Zhu K, Kakehi T, Matsumoto M, Iwata K, Ibi M, Ohshima Y, Zhang J, Liu J, Wen X, Taye A et al. (2015) NADPH oxidase NOX1 is involved in activation of protein kinase C and premature senescence in early stage diabetic kidney. *Free Radic Biol Med* **83**:21–30.

Address correspondence to: Andrea Cignarella, Department of Medicine, University of Padova, via Giustiniani 2, 35128 Padova, Italy. E-mail: andrea.cignarella@unipd.it
