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Review Article

Stress sensing within the breast tumor microenvironment: how glucocorticoid receptors live in the moment

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The classification and treatment of breast cancer is largely defined by the expression of steroid hormone receptors (HRs), namely estrogen receptor (ER) and progesterone receptor (PR), and gene amplification/overexpression of human epidermal growth factor receptor 2 (HER2). More recently, studies of androgen receptor (AR), glucocorticoid receptor (GR), and mineralocorticoid receptor (MR) have revealed that targeting these related HRs may be a promising strategy for a more personalized approach to the treatment of specific subtypes of HR+ breast cancer. For example, GR expression is associated with a good prognosis in ER+ breast cancer, but predicts poor prognosis in triple-negative breast cancer (TNBC). GR, like ER, PRs, and AR, is a ligand-activated transcription factor, but also has significant ligand-independent signaling activities. GR transcriptional activity is classically regulated by circulating glucocorticoids (GCs; ligand-dependent). Recent studies demonstrate that GR transcriptional activity is also regulated by a variety of cellular stress stimuli that input to GR Ser^{134} phosphorylation via rapid activation of the p38 mitogen activated protein kinase (MAPK) signaling pathway (ligand-independent). Furthermore, ligand-independent GR activation promotes feedforward signaling loops that mediate sustained activation of stress signaling pathways to drive advanced cancer biology (i.e. migration, invasion, chemoresistance, survival, and cellular growth). In this review, we will focus on the role of GR as a key sensor and mediator of physiologic and tumor microenvironment (TME)-derived cellular stress signaling in TNBC and discuss how targeting GR and/or associated signaling pathways may provide a strategy to inhibit deadly TNBC progression.

Introduction

Breast cancer affects up to 12.5% of American women in their lifetime [1]. It is the second leading cause of cancer-associated deaths in women in the United States [1]. Basic and translational cancer research has significantly improved outcomes for the majority of breast cancer patients [2]. Namely, the discovery of how steroid hormone receptors (HRs; estrogen and progesterone receptors (ER and PR)) contribute to pathogenesis and disease progression has provided insight into how to target and decrease progression of the major breast cancer subtypes. Clinicians primarily rely on the subtyping of breast cancer by identification of HRs and other surface receptors that are targetable with Federal Drug Administration (FDA)-approved agents. Treatment is largely dictated by using the following breast cancer subtyping: HR positive (+)/human epidermal growth factor receptor 2 (HER2) amplification negative (−), HR+/HER2+, HR−/HER2+, and HR−/HER2− [3,4]. Thus, approaches to breast cancer treatment are multidisciplinary and require the integration of surgery followed by a growing mix of neoadjuvant and adjuvant therapies

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(i.e. inhibition of hormone and/or signaling molecules, cytotoxic chemotherapy, immunotherapy, and others). For example, patients with metastatic ER+ breast cancer now receive CDK4/6 inhibitors in combination with aromatase inhibitors (AIs) or selective ER degraders (SERDs), such as fulvestrant.

The HR−/HER2− cohort is often referred to as triple-negative breast cancer (TNBC). TNBC has the worse prognosis of the breast cancer subtypes and the treatment of TNBC remains a significant challenge. Population-based studies have clearly shown that TNBC disproportionately affects young African American women [5–9]. Furthermore, obesity has also been linked to the increased incidence of pre-menopausal TNBC in African American women [5]. More recently social determinants of health, and chronic life stress have been investigated for their role in TNBC disparities (reviewed in [10]). While not all studies are consistent, chronic life stress in the form of racial discrimination and social isolation have both been shown to contribute to breast cancer incidence and mortality [11–15]. Importantly, animal models of chronic stress/social isolation have shown that chronic stress and stress signaling promotes breast cancer progression by influencing tumor biology. These studies may provide insight into novel strategies to mitigate the development of breast cancer and improve upon existing breast cancer treatments [16–19]. The nomenclature used to classify HR− breast cancer is somewhat erroneous because we nominate these breast tumors as HR− based solely on the measurement of expression of ER and the presence/absence of ER-induced PRs. A growing body of studies have addressed the possible actions of other HRs in ER+ breast cancer and TNBC. For example, the androgen receptor (AR) has been shown to be expressed in all breast cancer subtypes. Interestingly, AR was found have tumor suppressor functions and be an indicator of good prognosis in some ER+ patients [20] but worse prognosis for HR− patients (TNBC) patients (reviewed in [21]). Thus, identifying the roles of non-ER HRs in TNBC is of great importance since existing therapeutics can be rapidly employed to abrogate the actions of AR, glucocorticoid receptor (GR), or mineralocorticoid receptor (MR). The focus of this review is on exploring the role of GR as a key mediator of physiologic and tumor microenvironment (TME)-derived stress-induced signaling pathway activation that contributes to TNBC progression. We contend that GRs sense a convergence of host and cellular stress signaling to orchestrate an array of advanced cancer phenotypes associated with dangerous TNBC progression that could be targeted to improve patient outcomes.

Physiologic stress is an endocrine disruptor

Physiological stress and circadian rhythms regulate GR via the hypothalamus–pituitary–adrenal (HPA) axis. Stress hormones (i.e. glucocorticoids (GCs) or cortisol) are synthesized in response to acute and chronic life/host stress stimuli and the diurnal circadian rhythm. Adrenocorticotropic hormone (ACTH) is synthesized in the anterior pituitary and travels via the bloodstream to the adrenal glands where cortisol is produced (adrenal cortex) (Figure 1A). During a normal diurnal circadian rhythm, cortisol levels are lowest around midnight and then build to a peak around 7 a.m., each 24-h period. Chronically stressed individuals (i.e. low socioeconomic status or other stressful life circumstances such as social isolation or conditions of hardship or chronic pain, etc.) and those with altered sleep cycles, such as night shift workers, have been found to have disrupted cortisol circadian rhythms [22,23] (Figure 1A). A growing body of human population studies have linked stressful conditions such working over 55 h/week [24] as well as psychosocial stress including social isolation [16,17,19] to altered gene expression and increased risk of developing aggressive metastatic forms of breast cancer. In seminal studies performed in Sprague–Dawley rats [18], social isolation (i.e. one female per cage) was associated with low baseline or resting cortisol levels, but sustained elevation of cortisol levels following acute stress (i.e. temporary restraint); these changes had profound effects on mammary gland physiology. Isolated animals experienced greatly increased spontaneous mammary tumor burden (both number and size) and their tumors were highly invasive relative to grouped control subjects. Interestingly in humans, methylation of *NR3C1*, the gene that encodes GR, has been associated with an early life history of adverse childhood events, such as childhood abuse, psychological trauma, and anxiety [25,26]. It is unknown if these individuals experienced elevated breast or other cancer risk, but as noted above chronic life stress and social isolation have both been shown to contribute to breast cancer incidence and mortality [11–15]. Notably, in a small cohort of predominately ER+ patient samples NR3C1 methylation was observed in 15% samples and associated with decreased ER expression [27]. A subsequent study in ER+ breast cancer found that methylation of two different NR3C1 promoter regions were differentially associated with overall survival [28]. Additional studies are necessary to assess the effect of chronic life stress and the social determinants of health on methylation of NR3C1, altered cortisol cycles, expression or activity of GR, and the development of TNBC.

Responses to cortisol are highly tissue-specific and possibly determined by GR isoform or variant expression, the availability of HR cofactors, as well as the hormonal/signaling context in the form of direct or indirect

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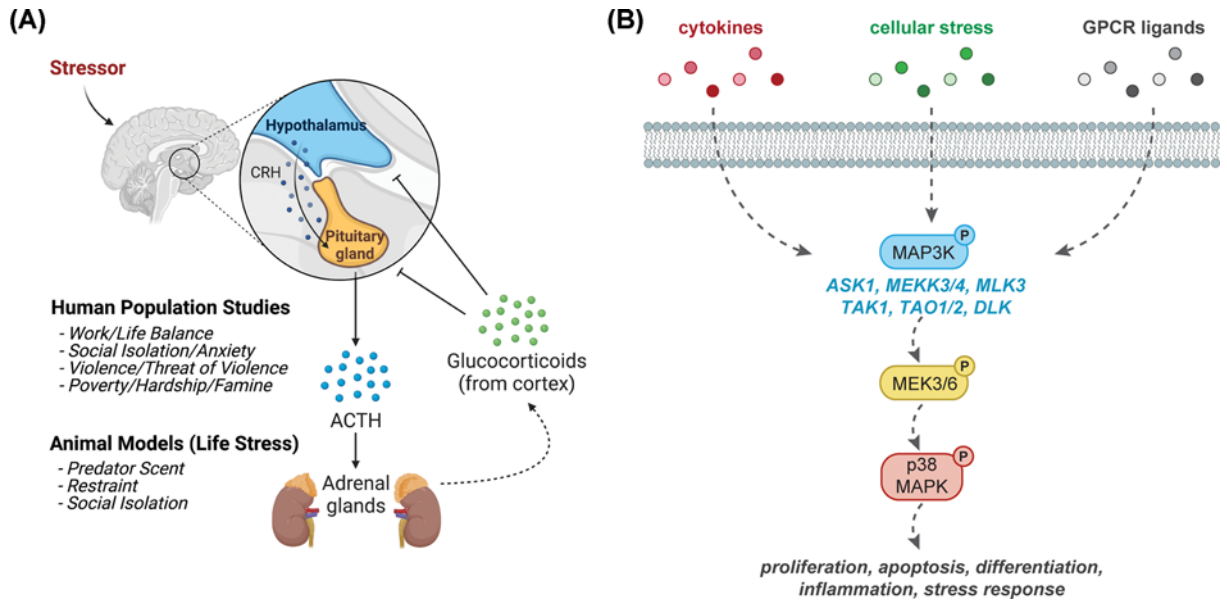

Figure 1. Life stress versus cellular stress stimuli to GR

(**A**) GC secretion by the HPA axis. Physiological/life stress induces the release of corticotrophin-releasing hormone (CRH) from the hypothalamus, which is then transported to the pituitary gland. This triggers the release of ACTH into the bloodstream and stimulates the adrenal cortex to synthesize and release GCs. GCs can then act on the hypothalamus and pituitary to reduce excess activation of the HPA axis (i.e., negative feedback) if necessary. Examples of life stress from human population and animal model studies are listed. (**B**) Schematic showing stress-activated signaling of p38 MAPK. Cytokines (e.g. TGFβ), cellular stress (e.g. reactive oxygen species [ROS]), or GPCR ligands lead to activation of MAP3Ks (ASK1, MEKK3/4, MLK3, TAK1, TAO1/2, DLK). This triggers phosphorylation of MAP2Ks (MEK3/6), which in turn phosphorylate and activate p38 MAPKs. Abbreviations: MAPK, mitogen activated protein kinase; TGFβ, transforming growth factor β.

post-translational modifications that alter GR activity. Aberrant changes to this tightly regulated homeostatic machinery can induce pathogenesis. Cancer cell signaling parallels cellular stress signaling for many of the pathways that drive cancer progression. GR is an important mediator of cellular/tissue responses to host stress in the form of corticosteroids. We and others have proposed that GR also acts as a 'local' sensor that links physiologic and cellular stress stimuli [29,30]. Cellular stress is induced by solid tumor core necrosis/hypoxia, tissue remodeling during angiogenesis, tissue damage and associated elevated oxygen tension/ROS, altered metabolism and the presence of abundant cytokines that contribute to activation of classical cellular stress-induced signaling pathways [31] (Figure 1B). For example, p38 and JNK mitogen activated protein kinases (MAPKs) are elevated or activated in numerous cancer subtypes including breast cancer [32–34]. These kinase families are collectively termed stress-activated protein kinases (SAPKs). They are also important in immunological processes and respond to paracrine factors including local cytokines that are abundant in the TME milieu (reviewed in [35]). Cytokines have been shown to directly and indirectly modulate the activity of the GR [36,37]. Ultimately, cellular stress is likely to be an important input to modulation of GR post-translational modification (i.e. phosphorylation) and altered transcriptional activity and/or promoter selection, thus providing a decisive means by which cells, including cancer cells, may respond to conditions of chronic cellular stress via regulation of changes in gene expression.

GRs are ubiquitously expressed ligand-activated transcription factors

The major GR isoforms (α and β) encoded by the *NR3C1* gene are well-studied members of the HR superfamily [38,39]. Numerous GR isoforms are generated from a single gene by alternative splicing and alternative translation initiation [40]. Over 1500 naturally occurring and stress-induced GR variants were recently detected in human peripheral blood; most of these GR variants reflect the presence of single nucleotide polymorphisms (SNPs) in human populations, but 21 unique slice isoforms containing at least 15 new cryptic exons were also detected [41]. Importantly, some NR3C1 SNPs are associated with increased GR transactivation potential [42]. While many SNPs and

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splice variants have been identified, few studies have examined the relationship between these variants and race or specific breast cancer patient populations. One study examined genetic variants in HPA axis genes, including four NR3C1 SNPs. In this case–control study of Caucasian and African American women, no significant associations were found after correcting for multiple comparisons [43]. Future studies that focus on breast cancer subtypes in specific human populations may reveal significant associations.

Similar to other steroid HRs, GRα (GR) structure includes an N-terminal transactivation domain (NTD), a DNA-binding domain (DBD), and a C-terminal ligand binding domain (LBD). In the absence of cortisol or other agonist ligand, GRs mostly reside in the cytoplasm of the cell. An activating-function (AF1) motif in the NTD region is essential for the binding of cofactors and other components that are required for transcription and translocation of GR to the nucleus (reviewed in [44]). In the presence of natural ligands, such as cortisol, GRs translocate to the nucleus and interact with DNA to regulate the transcription of gene sets important for cellular homeostasis such as reduction in inflammation, immunomodulation, and execution of the circadian rhythm (reviewed in [44,45]). Ligand-bound GRs utilize GC response elements (GREs) in DNA containing two pseudo-palindromic repeats of six bases: AGAACAnnnTGTCCG [46]. However, the mechanism by which GRs modulate gene transcription is complex involving both direct and indirect contacts with DNA. In a recently described model of GR action, three main mechanisms of gene regulation were described: (1) GRs can bind to GR-binding sites that have little agonist dependency for accessibility to chromatin (ligand-independent), (2) GRs also bind to sites occupied by pioneering factors, such as BRG1, in either the presence or absence of agonist, and (3) ligand-bound GRs may regulate gene expression via induction of extensive chromatin remodeling [44,47,48]. In addition to these mechanisms, the GR transcriptome is highly tissue-specific. Importantly, recent advances in the field of single-cell genomics, has identified that GR-induced transcription is also heterogeneous at the level of individual cells, with the average hormone-treated breast cancer cell exhibiting a transcriptional response at a minority (∼30%) of GR target genes [49]. Thus, transcriptional activity of GR is not only tissue-specific but also highly dependent on the cellular context. The mechanisms of how GR accomplishes this level of specificity and functional heterogeneity are yet to be fully defined. Although GRs depend on binding to agonist ligands for many classical activities, there is growing evidence that GRs also function beyond agonist-dependent activation of transcription. Although an understudied area of investigation relative to what is known about ER or PR rapid signaling [50], GR can also signal through non-genomic signaling pathways. Notably, GR has been shown to interact with c-Src and influence its tyrosine kinase activity in the cytoplasm [51]. This likely happens with the cooperation of other signaling and/or scaffold proteins that reside in the cytoplasm, such as HIFs or PELP1 [52–54]. Ultimately, as with other HR family members (namely ER and PR), a combination of both non-genomic and genomic GR actions, primarily described in neurons [55], most likely also occurs in breast cancer cells [56] and may in part underlie tumor heterogeneity.

The GR is a ubiquitous HR that is overexpressed in many cancer types [57]. Notably, GR is highly expressed in endocrine-related neoplasia such as prostate, ovarian, pancreas, and breast cancers [57]. Much work is needed to better define the role of GR in these cancer types. However, substantial evidence implicates GR in breast cancer biology and clinical prognosis. Importantly, GR has a dichotomous role in breast cancer depending on subtype and context (reviewed in [30]). Recent studies have suggested that GR predicts good outcome in breast cancer patients whose tumors are ER-positive [58]. In this context, GR may interfere or compete with ER and/or PR at the genomic level for the regulation of genes that are required to fuel ER-positive breast cancer progression. For example, GR direct interference with PR genomic regulation has been reported in ER+ breast cancer cells [59]. Notably, immune cell infiltrates significantly contribute to GR expression in breast tumors [60]. GR restrains inflammatory processes via repression of TNF-regulated genes and by transcriptional cooperation with NF-κB signaling [61]. However, in more advanced ER+ breast cancers, GR blockade of E2-induced apoptosis occurs via suppression of NF-κB signaling (i.e. loss of NF-κB phosphorylation), as reported in long-term E2-deprived models [62]. In contrast with luminal breast cancer, overexpression of GR predicts poor prognosis in TNBC patients [58]. GR-driven actions in the context of TNBC are still poorly understood. Several reports have established that GR confers increased growth and chemotherapy resistance in TNBC in the presence of chemotherapeutic agents such as paclitaxel (taxane-based chemotherapeutic agent) [52]. Recently, an activated-GR gene signature was identified in TNBC patients that modulated the expression of genes essential for processes such as tumor invasion, cancer cell survival, cellular transformation, epithelial–mesenchymal transition (EMT), and metastasis [63]. Thus, GRs contribute to cancer metastasis in addition to chemotherapy resistance. For example, GR activation with the artificial ligand Dexamethasone (Dex) increased metastasis in mouse models [64]. Interestingly, these models also showed increased expression of key kinases involved in breast cancer progression [64]. Another report demonstrated that EMT is regulated by GR via suppression of insulin-receptor substrate-1 (IRS-1) and activation of ERK2 MAPK [65]. Thus, the oncogenic actions of GR expressed in TNBC are in part mediated by cytoplasmic protein kinases with high cancer relevance. Cytoplasmic GR and concomitant loss

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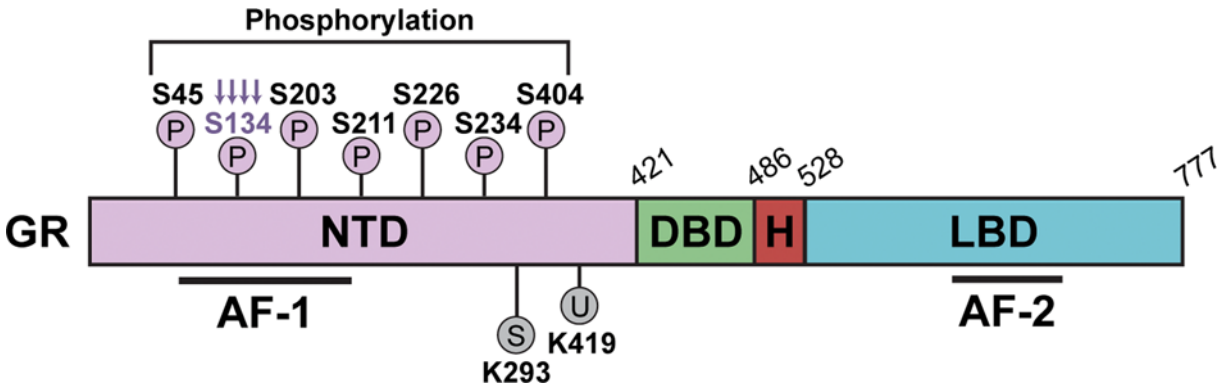

Figure 2. Domain structure and post-translational modifications of GR-α

GR domain structure contains: NTD, DBD, and C-terminal LBD. Post-translational modifications of GR include phosphorylation (Ser^{45}, Ser^{134}, Ser^{203}, Ser^{211}, Ser^{226}, Ser^{234}, Ser^{404}), sumoylation (Lys^{293}), and ubiquitylation (Lys^{419}).

of COX2 expression was associated with breast cancer development as measured by immunohistochemical analyses of a continuum of benign vs malignant breast tissue samples [66]. These studies suggest that GR location may differ in luminal vs TNBC; we reported less total GR, but increased levels of p-Ser^{134}-GR that was also more cytoplasmic in TNBC relative to luminal BC cases [52]. Altered GR localization and changes in GR post-translational modification may contribute to the recently reported heterogeneity of GR functions within individual breast cancer cells [49]. Notably, related MR are predominantly cytoplasmic in TNBC [67] and may interact with cytoplasmic and/or activated phospho-GRs (reviewed in [68]).

Additional reports in TNBC models have revealed interactions of GR with other steroid HRs such as MRs (noted above) or ARs [69]. Interestingly, TNBCs frequently express both AR and GR. As in luminal breast cancer cases, co-expression of these HRs correlates with improved overall and disease-free patient survival. However, high expression of GR and low expression of AR predict decreased overall survival in ER-negative patients. It is possible that when AR is present, GRs compete for limiting HR cofactors and are thus less able to influence the expression of genes required for cancer progression (i.e. EMT, invasion, chemoresistance etc) [69]. Similar processes either limit (i.e. by competition) or promote ER functions when other HRs are co-expressed in luminal breast cancer (mentioned above). Not surprisingly, as in luminal breast cancer, dynamic changes in the levels and activities of multiple co-expressed HRs as well as signaling pathways during tumor progression can dramatically alter TNBC responses to therapies [70]. Linkage of HR action to known biomarkers (i.e. signaling readouts including the presence of p-HRs and expression of their target genes) may help guide therapeutic strategies.

GR post-translational modifications with cancer relevance

A variety of post-translational modifications to GR have been well-characterized. Similar to other HR family members, GR is modified by phosphorylation, ubiquitylation, sumoylation, acetylation, and nitrosylation (reviewed in [44]). GR Lys^{419} ubiquitylation tags transcriptionally active receptors for nuclear export and rapid ligand-dependent protein turnover via the 26S proteasomal pathway [71]. Sumoylation events largely stabilize GR and attenuate transcriptional activity (reviewed in [44]). Specifically, sumoylation of GR Lys^{293} leads to transrepression of GR transcriptional activity by inducing the recruitment of corepressors such as the silencing mediator for retinoid or thyroid HR (SMRT) or the nuclear receptor corepressor 1 (NCoR1) [72]. GR acetylation has been shown to alter GR sensitivity to agonists such as Dex. Histone deacetylase 2 (HDAC2) is responsible for deacetylating GR, a process that induces its interaction with NF-κB and subsequently regulates gene expression [73]. Interestingly, ligand insensitivity is caused by GR nitrosylation, which induces displacement of the zinc molecules held within the zinc finger DNA-binding domains and thereby inhibits ligand interaction (reviewed in [44]). Phosphorylation of GR is well-studied and has been reported to occur on at least seven residues (S113, S134, S141, S203, S211, S226, and S404) located in the GR NTD (Figure 2). In 1997, Cidlowski et al. showed that the transcriptional activity of mouse GR largely depends on these phosphorylation sites [74]. The same report [74] showed that these sites also influence the half-life of the GR, suggesting coordination of selected phosphorylation events with ubiquitinylation and/or sumoylation, as has been reported for related HRs, such as PR [75,76].

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Given the well-established role of GR modulation by phosphorylation events (reviewed in [50]), it is important to define how GR is regulated in the context of altered signaling pathways that typify cancer. Diverse signaling inputs have been shown to impact GR phosphorylation on multiple sites. Interestingly, robust phosphorylation primarily occurs in the presence of GR agonists (reviewed in [44]). An exception to this is regulation of GR Ser^{134} phosphorylation by p38 MAPK, which occurs in the absence of GR ligands. Phosphorylation of GR Ser^{134} mediates interaction with the cytoplasmic scaffolding protein 14-3-3ζ (Figure 3) [36]. The 14-3-3 family of proteins act as molecular chaperones that primarily modulate cellular signaling [36]. Often, these proteins are essential for survival and they are known to modulate the action of MAPKs. Notably, 14-3-3 proteins have been implicated in breast cancer progression. Specifically, 14-3-3ζ promoted anchorage-independent growth and halted apoptosis in breast cancer models [77]. Additionally, 14-3-3ζ is an important mediator of transforming growth factor β1 (TGFβ1) signaling; in response to TGFβ1, 14-3-3ζ stabilized GLi2 and in turn promoted the action of SMAD partners via direct binding to drive metastasis from breast tissue to bone [78]. Using a tissue microarray representing 281 diverse primary breast cancers, we showed that p-Ser^{134}-GR is elevated in TNBC relative to non-TNBC subtypes, suggesting that pS134-GRs acquire unique oncogenic functions in this disease, possibly in concert with p38 MAPK [52]. Accordingly, potent cellular stress stimuli (hypoxia, reactive oxygen species, nutrient deprivation) including cytotoxic chemotherapies (paclitaxel, 5-fluorouracil; 5-FU) and loss of cell attachment (i.e. 3D culture) promoted GR Ser^{134} phosphorylation via activation of p38 MAPK in both luminal and TNBC models [52]. Inducible p-S134-GR target genes included PTK6, also known as breast tumor kinase (Brk), HIF-2α, and PELP1 [53]. We subsequently defined p-GR/HIF1-2/PELP1 transcriptional complexes in cooperation with the aryl hydrocarbon receptor (AhR) at known xenobiotic stress-induced AhR target genes in TNBC models (Figure 4) [52]. Additionally, cytokines (IL6, TGFβ) are potent inputs to p38 MAPK-dependent GR Ser^{124} phosphorylation [79]. The ligand-independent pS134-GR transcriptome encompassed both TGFβ1 and MAPK signaling gene sets (including MAP3K5/ASK1) linked to TNBC cell survival and migration/invasion. Accordingly, we showed that pS134-GR and 14-3-3ζ were essential for TNBC cell anchorage-independent growth in soft agar, migration, invasion, and tumorsphere formation, an *in vitro* readout of cancer stemness properties. Surprisingly, both pS134-GR and expression of 14-3-3ζ (discussed above) were essential for a functionally intact p38 MAPK signaling pathway downstream of MAP3K5/ASK1, indicative of a feedforward signaling loop wherein self-perpetuated GR phosphorylation enables pernicious cancer cell autonomy in response to cellular stress (Figure 3). These findings nominate p-Ser^{134}-GR and 14-3-3ζ as essential effectors of p38 MAPK signaling and underscore that unliganded but activated GRs, via phosphorylation of Ser^{134}, are able to sense a diverse array of inducible inputs that are involved in cellular responses to stress and relevant to cancer processes such as EMT, formation of stem cells, altered cancer metabolism, increased mobility, and ultimately metastasis.

GR target genes are useful biomarkers and potential novel drug targets

Gene signatures indicative of activated GRs have great potential as diagnostic biomarkers. A 24-gene pS134-GR-dependent signature induced by TGFβ1 predicted shortened overall survival in breast cancer patients regardless of subtype. Indeed, the stress-induced phospho-GR 'trigger' described above is an attractive reversible pathway that could be targeted therapeutically. Additionally, several ligand-induced GR target genes with clear cancer relevance have been identified in TNBC, many of which are important for cancer processes associated with chemotherapeutic resistance. For example, both SGK-1 and MKP-1 are Dex-induced mediators of cancer cell pro-survival and resistance to chemotherapy [80]. Ectopic expression of either SGK-1 or MKP-1 enables resistance to paclitaxel (taxane-based chemotherapy) or doxorubicin in multiple breast cancer cell lines [80]. Notably, both of these proteins are tightly linked to the MAPK family. SGK-1 is a serine/threonine kinase downstream of the PI3K pathway, which can indirectly modulate the action of MAPKs' modules (reviewed in [81]). Recent data indicate that GRs mediate metastasis in part via the expression of ROR1 [64], a transmembrane protein tyrosine kinase that modulates cellular growth. In the context of TNBC, ligand-dependent activation of GR increased the expression of ROR1 *in vivo*. ROR1 gene amplification is correlated with decreased breast cancer patient survival and may represent a useful clinical biomarker [64].

Studies elucidating the role of GR in TNBC have led to the discovery that numerous protein kinases associated with cellular stress signaling (i.e. p38 MAPKs discussed above) are involved in GR-driven breast cancer progression. Protein tyrosine kinase 6 (PTK6; also known as breast tumor kinase or Brk, mentioned above) is an intracellular soluble tyrosine kinase that promotes phosphorylation of signaling molecules (Rac1, ERK5, p38 MAPKs) as well as factors involved in transcription (STATs, MEFs) and RNA splicing (SAM68). PTK6 has both cytoplasmic and nuclear actions and its SH2/SH3 domain structure is distantly related to the c-Src kinase family with greatest homology to the c-Src

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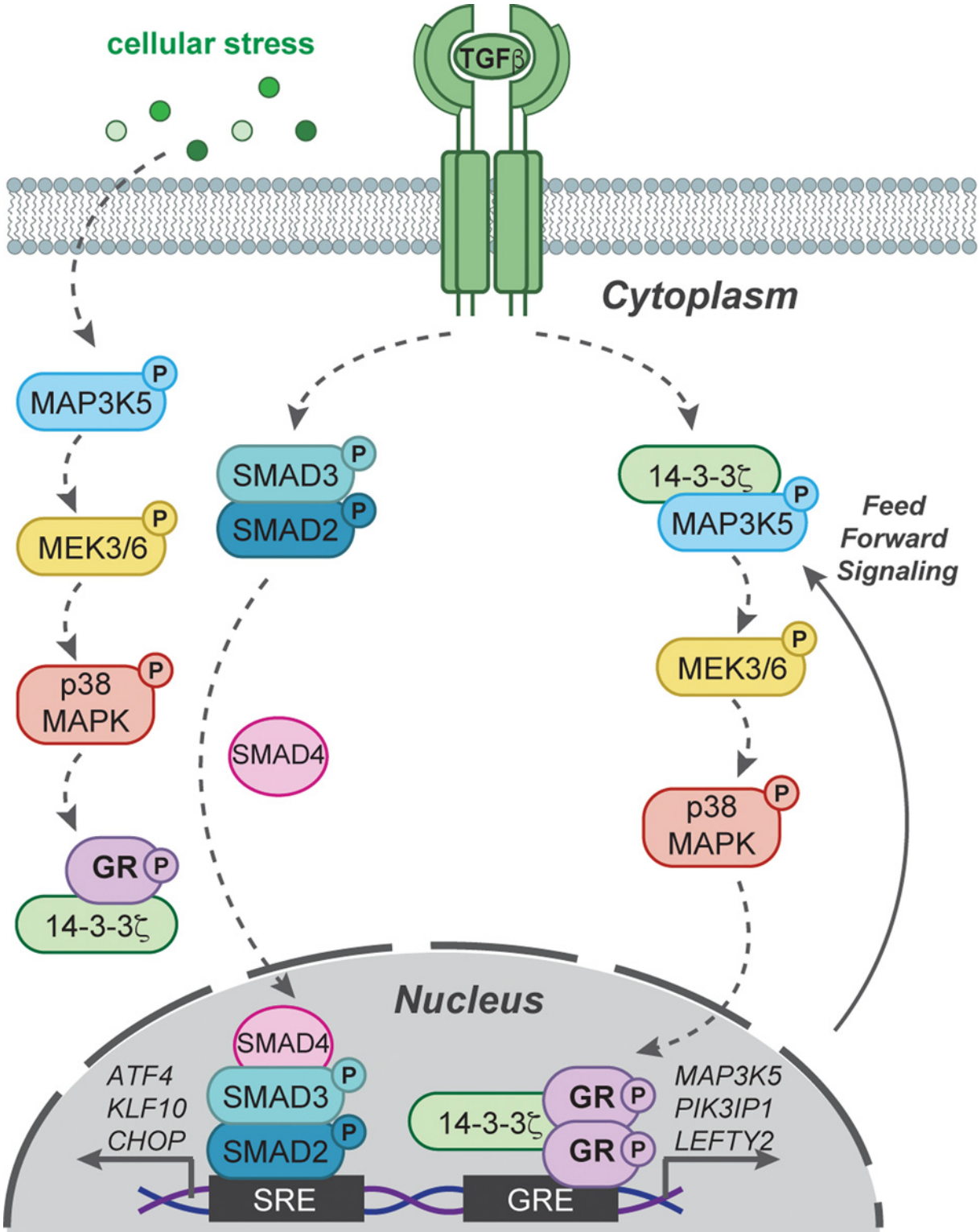

Figure 3. Phospho-GR (Ser^{134}) participates in a feedforward signaling loop that further activates the p38 MAPK pathway downstream of TGFβ1 in TNBC models

TGFβ1 activates SMADS, but can also activate the p38 MAPK module (i.e. MAP3K5, MEK3/6, and p38 MAPK) to phosphorylate GR (Ser^{134}). Ligand-independent phospho-GR (Ser^{134}) target genes include key components of the p38 MAPK pathway (MAP3K5) needed for intact p38 signaling (feedforward signaling). Cellular stress can also induce phosphorylation of GR at Ser^{134} via the p38 MAPK pathway.

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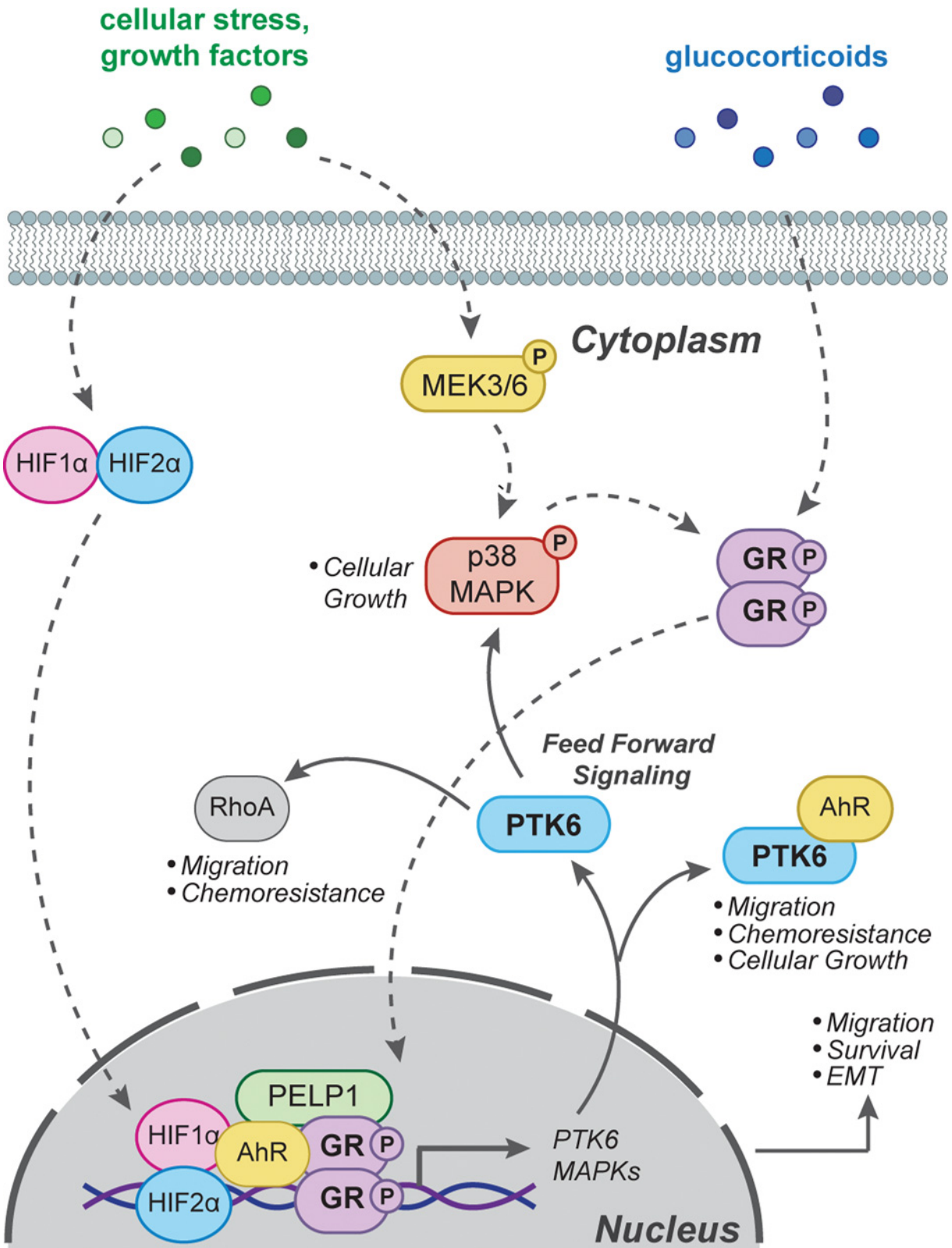

Figure 4. Phospho-GR/HIFs regulates PTK6 expression to drive a feed forward signaling loop

Stress stimuli stabilize HIFs and also activate p38 MAPK, which leads to ligand-independent phosphorylation of GR (Ser^{134}). Phospho-GR (Ser^{134}) forms a transcriptional complex with HIFs, PELP1, and AhR to induce PTK6 expression. In turn, PTK6 participates in a feedforward signaling loop to p38 MAPK. Additionally, the SH2 domain of PTK6 is integral in regulation of RhoA and AhR oncogenic activity.

kinase domain [82]. PTK6 is a potent mediator of breast cancer cell migration, pro-survival, and chemotherapeutic resistance [83]. Paclitaxel or 5-FU promote phosphorylation of GR on S134 to induce sustained PTK6 mRNA and protein expression [52]. Recently, the PTK6 SH2 domain (i.e. pTyr-binding) was shown to be required for activation of p38 MAPK, AhR, and Rho signaling, events that occurred independently of the PTK6 kinase domain in TNBC models [84]. Taken together, these studies illustrate that GR actions are highly integrated with cellular stress-induced signaling pathways and their key mediators.

Given that GR/p-GR is emerging as a potent and inducible driver of TNBC and potentially other highly aggressive cancers, it is time to rethink the clinical use of GCs (GR agonists) as palliative agents. Dex is a GC that is widely administered to patients previous to receiving chemotherapy or radiotherapy for treatment of breast cancer and other cancer types. Patients are prescribed high-dose Dex or other GCs to engage the anti-inflammatory actions of GR in order to minimize adverse side effects such as nausea and vomiting post-chemotherapy [85]. It is possible that both GR expression and unwanted actions of GR (i.e. induction of anti-apoptotic or pro-survival pathways, cancer cell migration/invasion, and metastasis) are being inadvertently induced by these agents and thereby rendering cancer therapies less effective. Of note, GR and GR target genes (PTK6) were robustly induced following a single dose of Dex treatment of human tumor explants cultured on gelatin sponges [53] as well as in adrenalectomized mice, as measured in their mammary glands only 6hr after intraperitoneal injection [52]. More studies are urgently needed to address this clinically relevant scientific question and the benefits and risks associated with GC treatment in patients receiving chemo- or radiotherapy. In contrast with GR activation, targeting GR (i.e. with GR antagonists) or p-GR (i.e. with kinase inhibitors) may provide a highly effective means to cripple potent epigenetic drivers of dangerous TNBC progression. Of note, HRs rely on heat shock protein (Hsp) molecular co-chaperones for proper folding, stability, and trafficking [86]. Hsp90 inhibitors (ganetespib and NVP-AUY922) that induce GR degradation improve responses to paclitaxel in TNBC models [87]. Similarly, GR degradation induced by the FDA-approved asthma drug, ciclesonide, blocked cancer stem cell formation in MDA-MB-231 cells, experimental models of TNBC [88]. A dual AR/GR antagonist (CB-03-10) effectively blocked both prostate cancer and TNBC (MDA-MB-231) xenografts via induction of apoptosis [89]. Numerous case reports have described the success of RU486 (mifepristone) for treatment of both female and male patients with diverse cancers [90–93]. Clinical trials using GR antagonists to combat TNBC and other GR+ cancers are on the horizon [94]. These encouraging developments suggest that we can proceed with optimism that GR-blocking agents have great clinical potential for treatment of TNBC and other cancers for which GR/p-GR may be a driver of cancer cell survival and dissemination to metastasis.

Summary

- GR is a sensor of physiologic and TME-derived stress signals.
- GR post-translational modifications impact GR stability and transcriptional activity.
- Ligand-independent GR activation via phosphorylation promotes feedforward signaling loops that mediate sustained activation of stress signaling pathways to promote advanced cancer biology.
- GR antagonists are in clinical trials and have great clinical potential for the treatment of TNBC and possibly other cancers where GR is a driver of cancer cell survival and metastasis.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

All authors contributed to the writing and editing of the manuscript.

Abbreviations

AhR, aryl hydrocarbon receptor; AR, androgen receptor; COX2, cyclooxygenase 2; Dex, dexamethasone; E2, estradiol; EMT, epithelial–mesenchymal transition; ER, estrogen receptor; FDA, Federal Drug Administration; GC, glucocorticoid; GR, glucocorticoid receptor; HER2, human epidermal growth factor receptor 2; HIF, hypoxia inducible factor; HPA, hypothalamus–pituitary–adrenal; HR, hormone receptor; Hsp, heat shock protein; MAPK, mitogen activated protein kinase; MR, mineralocorticoid receptor; NTD, N-terminal transactivation domain; PELP1, proline, glutamic acid, leucine rich protein 1; PR, progesterone receptor; SNP, single nucleotide polymorphism; TGFβ1, transforming growth factor β1; TME, tumor microenvironment; TNBC, triple-negative breast cancer; 5-FU, 5-fluorouracil.

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