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G protein-coupled receptor kinase 2 (GRK2) as a potential modulator of the hallmarks of cancer

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Running title: GRK2 as an oncomodulator

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Abbreviations: CXCR4, C-X-C chemokine receptor type 4; EC, Endothelial cells; EGFR, Epidermal growth factor receptor; EPAC, Exchange factor directly activated by cAMP; ERM, Ezrin-Radixin-Moesin; GIT-1, GRK interactor 1; GRK2, G-protein-coupled receptor kinase 2; HDAC6, Histone deacetylase 6; IRS1, Insulin receptor substrate 1; MAPK, Mitogen-activated protein kinases; PAK, p21-activated kinase; PAR, Protease-activated receptors; PDE,Phosphodiesterase; Pin1, peptidyl-prolyl cis-trans isomerase NIMA-interacting 1; RKIP, Raf kinase inhibitor protein; S1P, Sphingosine-1-phosphate

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ABSTRACT

Malignant features such as sustained proliferation, refractoriness to growth suppressors, resistance to cell death or aberrant motility and metastasis can be triggered by a variety of mutations and signaling adaptations. Signaling nodes can act as cancer-associated factors by cooperating with oncogene-governed pathways or participating in compensatory transduction networks to strengthen tumor properties. G-protein-coupled receptor kinase 2 (GRK2) is arising as one of such nodes. Via its complex network of connections with other cellular proteins, GRK2 contributes to the modulation of basic cellular functions such as cell proliferation, survival or motility, and is involved in metabolic homeostasis, inflammation or angiogenic processes. Moreover, altered GRK2 levels are starting to be reported in different tumoral contexts and shown to promote breast tumorigenesis or to trigger the tumoral angiogenic switch. The ability to modulate several of the hallmarks of cancer puts forward GRK2 as an onco-modifier, able to modulate carcinogenesis in a cell-type specific way.

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Introduction

Cancer is caused by sequential genetic and epigenetic alterations that disrupt the regulatory circuits governing normal cell proliferation and homeostasis. This multi-step pathological process involves the progressive occurrence of molecular modifications and cellular adaptations by selective pressure during many years, what leads to considerable heterogeneity and variability among tumors. However, most tumors share essential alterations in cell physiology, which together promote malignant transformation and are termed “the hallmarks of cancer" (Hanahan and Weinberg, 2011). These hallmarks include maintained proliferative signaling, insensitivity to growth-inhibitory signals, resistance to cell death, limitless replicative potential, induction of angiogenesis, tissue invasion and metastasis, metabolic reprogramming and evading immune response. In addition, two transversal characteristics are permissive for the acquisition of these tumoral traits: development of genomic instability and the chronic inflammation linked to pre-malignant and tumoral lesions. Indeed, contributions of non-tumoral components (extracellular matrix, fibroblasts, resident immune cell types or vascular cells) are key for cancer progression and the interactions in the ‘‘tumor microenvironment’ play an increasingly important role in tumor biology.

Oncogene-driven proliferation and survival of tumor cells is assisted by various mechanisms. Cells can secrete their own growth factors (GF) or stimulate their release by cells within the stroma (Bhowmick *et al.*, 2004). Alternatively, GF-receptors can also be mutated or over-expressed, rendering cells hyper-responsive even to limited amounts of these ligands. Constitutive activation of signaling components (kinases, G protein switches, transcription factors)

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operating downstream to these receptors may also drive GF-independent proliferation (Hanahan and Weinberg, 2011) and are common targets for oncogenic transformation.

In addition, other non-genetically altered factors can cooperate with oncogenic routes or trigger compensatory pathways in order to strengthen tumoral properties or to cope with intrinsic tumor vulnerabilities. Regulatory molecular nodes able to integrate multiple upstream inputs and trigger diverse downstream outputs are particularly suitable to act as non-oncogenic contributors to malignant transformation and progression. In this context, G-protein-coupled receptor kinase2 (GRK2) is emerging as a potentially relevant onco-modulator, given its functional connections with the most relevant signaling networks required for the proper function, homeostasis and viability of the cell. In this review, we recapitulate the results linking GRK2 to several pathways related to the hallmarks of cancer (Fig. 1) and emerging evidence pointing to a role for this kinase in the progression of specific tumors.

Canonical and non-canonical roles of GRK2

G-protein-coupled receptor kinases (GRKs) were first described as negative GPCR modulators. Agonist-occupied GPCR become specifically phosphorylated by these kinases (Gurevich *et al.*, 2012), thus allowing the recruitment of β-arrestins, leading to uncoupling from G proteins and GPCR internalization. However, arrestins are scaffold proteins for several signaling mediators, eliciting additional signaling pathways from GPCR signalosomes (Shenoy and Lefkowitz, 2011). Therefore, changes in GRK functionality could also influence the balance/bias between the G protein-dependent and GRK/β-

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arrestin-branches of GPCR signaling and the recruitment of arrestin interactors (Penela, Murga, *et al.*, 2010).

GRK2 is a ubiquitous member of the GRK family. Global GRK2 knockout mice are the only among the GRK family that are embryonically lethal, indicating a critical role in core cellular processes. Consistently, research in different laboratories has shown that GRK2 can also participate in signaling networks by directly interacting and/or phosphorylating non-GPCR cellular partners (Penela, Murga, *et al.*, 2010; Evron *et al.*, 2012; Gurevich *et al.*, 2012), including receptor-tyrosine kinases (RTKs) and a variety of cytosolic or nuclear signaling proteins (see below). A main challenge is to gain further insight on how these varied canonical and non-canonical GRK2 functions integrate in specific cell types and contexts and at the organism level, and to discern whether the GRK2 signaling hub can be altered and contribute to disease progression in cancer or other pathological conditions. The potential mechanisms by which GRK2 may participate in the pathways controlling the hallmarks of cancer are summarized in the following sections.

GRK2 regulates cell proliferation and survival cascades

Changes in GRK2 levels or functionality have been reported to affect MAPK/ERK activation and cell proliferation in different ways, depending on the cell type and mitogen stimuli involved. The mechanisms underlying such effects can be varied, including “canonical” desensitization of G protein-dependent MAPK stimulation by GPCR, modulation of GPCR-β-arrestin-MAPK cascades or of GPCR crosstalk with EGFR or other GF receptors (Filardo *et al.*, 2008; Sosa *et al.*, 2010). In addition, GRK2 is able to phosphorylate and/or interact with relevant components of the MAPK pathway downstream the stimulation of

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GF receptors or of mitogenic GPCRs, such as GIT-1, RhoA, Epac, PDEγ, RKIP or Pin1 (Wan *et al.*, 2003; Penela *et al.*, 2008; Penela, Rivas, *et al.*, 2010; Deiss *et al.*, 2012; Robinson and Pitcher, 2013; Singhmar *et al.*, 2016).

GRK2 promotes MAPK activation and/or cell proliferation triggered by some tumor-related GPCRs. In epithelial cells, GRK2 potentiates MAPK stimulation by the lipid S1P1 receptor by associating to GIT1 (Penela *et al.*, 2008), whereas it fosters β-arrestin-MAPK activation by the chemokine receptor CXCR7 in astrocytes (Lipfert *et al.*, 2013). GRK2 also potentiates Hedgehog/Smoothened-mediated transformation in fibroblast cell lines (Meloni *et al.*, 2006; Zhao *et al.*, 2016). In this context, the stimulatory effect of GRK2 in cell proliferation during early embryonic development involves the interaction of GRK2 with Patched, thus relieving the Patched-induced cytosolic retention of cyclin B in response to Hedgehog (Jiang *et al.*, 2009). In addition, GRK2 enhances MAPK signaling in response to integrins in epithelial cells and fibroblasts (Penela *et al.*, 2008), and to EGF in HEK-293 cells (Wan *et al.*, 2003), vascular smooth muscles cells (Robinson and Pitcher, 2013), or epithelial cells (Penela *et al.*, 2012; Nogués *et al.*, 2016). IGF-1-triggered proliferation and mitogenic signaling in osteoblasts is dependent on GRK2 kinase activity (Bliziotes *et al.*, 2000). Conversely, GRK2 weakens serum- or PDGF-induced proliferation of thyroid cancer (Métayé *et al.*, 2008) and smooth muscle cells (Peppel *et al.*, 2000), as well as IGF1-dependent proliferation of human hepatocellular carcinoma (HCC) (Wei *et al.*, 2013) or HEK-293 cells (Zheng *et al.*, 2012; Fu *et al.*, 2013) likely via desensitizing phosphorylation of these GF receptors.

The reported functional interaction of GRK2 with several proteins involved in the cellular response to stress, including p53, p38, Smad2/3, AKT, Hsp90 or

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HDAC6 (reviewed in Penela, Murga, *et al.*, 2010; Lafarga *et al.*, 2012), might modulate cell survival and resistance to apoptosis in certain contexts. GRK2 accumulates in the presence of DNA damaging agents that activate cell cycle arrest such as doxorubicin, thus contributing to counterbalance the stimulation of the p53 pathway elicited by G2/M checkpoint mechanisms and preventing apoptosis of arrested cells (Penela et al., 2010). p38, which can either promote apoptosis or survival depending on the cell type and pathological context, is phosphorylated by GRK2, what prevents binding of upstream activators and certain substrates (Peregrin *et al.*, 2006). In endothelial cells,GRK2 has been reported to inhibit Akt via direct association between these kinases (Liu *et al.*, 2005; Horinouchi *et al.*, 2016), whereas a positive effect of GRK2 on this pathway via PI3kgamma interaction has been shown in cardiomyocytes (Perrino *et al.*, 2007). Upon stimulation of the ALK5 receptor by TGFβ, GRK2 binds to and phosphorylate Smad2/3, preventing the nuclear shuttling of the Smad complex, thus hindering pro-apoptotic TGFβ effects and potentially enhancing its tumor-promoting role (Ho *et al.*, 2005, 2007).

GRK2 modulates immune and epithelial cell motility

GRK2 plays an important role in the migration of different cell types (reviewed in Penela *et al.*, 2014). GRK2 has been shown to trigger desensitization of a variety of chemokine receptors, therefore finely tuning chemokine-dependent signaling in lymphocytes and neutrophils during inflammation (Vroon *et al.*, 2006). Similarly, mobilization of macrophages to CCL5 is enhanced in GRK2 hemizygous mice, and LPS reportedly favors CCL2-induced macrophage migration by decreasing GRK2 levels and therefore avoiding CCR2 receptor

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desensitization (Liu *et al.*, 2013; Otten *et al.*, 2013; Rivas *et al.*, 2014). However, in particular contexts GRK2 down-modulation can show opposite effects in the migration of certain types of immune cell types to specific stimuli (Arnon *et al.*, 2011).

On the other hand, GRK2 is a positive player in epithelial cell migration via different mechanisms. GRK2 phosphorylates ezrin and radixin fostering its ability to modulate actin remodeling, migration and invasion (Cant and Pitcher, 2005; Kahsai *et al.*, 2010). GRK2 can also trigger polarity persistence in the presence of chemotactic messengers and integrins by increasing the intensity and duration of MAPK stimulation and the turnover of focal adhesions via the GIT-1 hub (Penela *et al.*, 2008). GIT1 is a multi-domain protein able to scaffold different partners involved in cell migration at focal adhesions and the cell leading edge, resulting in localized MAPK and Rac/PAK stimulation (Hoefen and Berk, 2006). In response to either fibronectin or S1P, GRK2 associates to GIT1 at the leading edge, fostering GIT-1-dependent stimulation of Rac1, F-actin cortical remodeling and MAPK, and leading to increased migration (Penela *et al.*, 2008).

Finally, GRK2 is able to control microtubule dynamics through activation of HDAC6 (Lafarga *et al.*, 2012). HDAC6 is a cytosolic histone deacetylase type II over-expressed in a high proportion of breast cancers and other type of tumors, reported to modulate cell growth, motility and invasion by allowing dynamic acetylation-deacetylation of cortactin or tubulin, among other substrates (Duong *et al.*, 2008; Lee *et al.*, 2008; Aldana-Masangkay and Sakamoto, 2011). In response to EGF, GRK2 associates with and phosphorylates HDAC6, what enhances its alpha-tubulin deacetylase activity, thus resulting in local tubulin

deacetylation at the leading edge of migrating cells and enhanced motility (Lafarga *et al.*, 2012). Notably, the ability of GRK2 to phosphorylate HDAC6 relies on the previous phosphorylation of GRK2 at S670 by MAPK in response to external stimuli. Importantly, S670-GRK2 phosphorylation would simultaneously disrupt its interactions with competing cellular partners such as GPCR or GIT-1, whereas turning on the modulation of HDAC6 at defined locations within the cell. The dynamic modulation of the phosphorylation status and subcellular localization of GRK2 by specific stimuli would thus allow rapid switching of interacting partners, allowing the concerted action of GIT-1 signalosomes and of de-acetylated MTs in cortical polarity and membrane protrusion (Penela *et al.*, 2014).

Notably, a number of the GRK2 partners discussed above are important players in tumor cell invasion, including plasma membrane GPCRs (for ligands as S1P, chemokines or proteases), integrins or EGF receptors and cytoskeleton modulators such as RhoA, Rac1, or ERMs. Other potential GRK2 targets, as the chemokine receptors CXCR4 and CXCR7, are highly expressed in a range of tumors and play a role in metastasis, although their role in cancer progression is not fully understood. CXCL12, the CXCR4 ligand, is constitutively expressed at tumor and metastatic sites (O'Hayre *et al.*, 2014). CXCR7 also binds CXCL12 and triggers downstream pathways in a G protein-independent manner and/or by scavenging CXCL12 away from CXCR4. Whether changes in GRK2 dosage taking place during cancer progression can modify signaling/ migration/ invasion/ angiogenesis mediated by the CXCL12/CXCR4/CXCR7 axis or by other chemokines present in the tumor microenvironment is an interesting avenue for future research.

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GRK2 modulates key cell metabolism networks

Important metabolic changes/adaptations taking place in different cell types during cancer progression as a result of fluctuations in oxygen or nutrient availability, altered mitochondrial function and redox status, modulation of autophagy and proteostasis, rewiring of proliferation or differentiation networks or modifications of the microenvironment (Lehuede *et al.*, 2016). On the other hand, epidemiologic data suggest that patients with insulin resistance/obesity have a higher risk of developing several types of cancer (Klil-Drori *et al.*, 2016).

In addition to desensitizing GPCR controlling metabolic rate and energy expenditure, such as adrenergic receptors (Vila-Bedmar *et al.*, 2012), GRK2 has emerged as an important player in insulin resistance and obesity, and in mitochondrial function (Ciccarelli *et al.*, 2012; Vila-Bedmar *et al.*, 2015; Hullmann *et al.*, 2016). GRK2 acts as a negative modulator of insulin cascades by either interfering with insulin-Gq/11 signaling leading to GLUT4 translocation (Usui *et al.*, 2004) or by interacting and/or phosphorylating IRS1 (Garcia-Guerra *et al.*, 2010; Ciccarelli *et al.*, 2011). Moreover, GRK2 levels are elevated during insulin resistance in several tissues in high-fat diet (HFD)- murine models (Garcia-Guerra *et al.*, 2010; Lucas *et al.*, 2014). Decreasing kinase levels can prevent and revert development of an insulin resistance and obesity phenotype in vivo (Vila-Bedmar *et al.*, 2015). In addition, in stressed cardiac cells GRK2 can localize to the mitochondria upon ERK1/2 GRK2 phosphorylation and subsequent complex formation with Hsp90. Although a protective effect has been suggested (increased biogenesis and ATP production)(Fusco *et al.*, 2012), others have reported that mitochondrial GRK2 displays a detrimental

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effect by increasing superoxide levels and altering substrate utilization for energy production (Chen *et al.*, 2013; Sato *et al.*, 2015; Cannavo *et al.*, 2016). Whether changes in GRK2 levels/activity taking place in tumor cells can modulate metabolic networks or mitochondrial functions is an interesting issue for future research.

GRK2 roles in tumoral angiogenesis and in the pro-inflammatory tumor microenvironment.

Tumor microvasculature is usually highly angiogenic and leaky, displaying enlarged and dilated vessels lined with immature walls due to the loss of pericytes, leading to deficient blood supply. Subsequent hypoxia prompts secretion of different pro-angiogenic and pro-inflammatory factors, perpetuating aberrant vascularization and inflammation (Potente *et al.*, 2011). In such microenvironment, reciprocal interactions among tumor-associated vasculature, tumor-infiltrated immune cells and transformed cells drive cancer progression.

GRK2 is emerging as a signaling node integrating several pathways involved in endothelial cell activation and maturation, such as those mediated by S1P, VEGF, PDGF-BB and TGFβ1 receptors. Upon GRK2 down-modulation, the response of endothelial cells (ECs) to relevant angiogenic stimuli (VEGF, S1P, serum) is increased, altering the capability of these cells to organize into tubular structures, as well as disrupting the balance in the secretion of inflammatory and angiogenic factors. Moreover, decreased GRK2 levels also modify TGF-β signaling, which controls both the activation and resolution phases of angiogenesis via the timely modulation of the opposite effects of ALK1 and ALK5 receptors (Rivas *et al.*, 2013, 2014). Consistent with such impacts on

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endothelial cell signaling, GRK2 loss impedes ECs differentiation and fusion into tubular structures and hampers the recruitment of pericytes, leading to immature and leaky vessels (Rivas *et al.*, 2013). Remarkably, EC-cell specific GRK2 silencing fosters the growth of tumors in mice. The vessels of tumors developed in these animals display increased size and reduced pericyte recruitment, key features of the tumor microvasculature. Notably, endothelial GRK2 dosage is indeed down-regulated in human breast cancer vessels (Rivas *et al.*, 2013). Whether such tumor cell-driven endothelial cell GRK2 down-modulation applies or not to different types of cancer and the molecular mechanisms involved remain to be established.

Interestingly, endothelium-specific down-modulation of GRK2 also appears to promote the recruitment of macrophages to the tumor by directly altering the chemotactic secretome of ECs and indirectly by promoting leaky vessels, leading to a gradient of hypoxia-induced chemoattractants for myeloid cells (Rivas *et al.*, 2013). Since GRK2 is a well-established modulator of chemokine receptors in immune cells in human inflammatory diseases (Vroon *et al.*, 2006), is tempting to speculate that changes in GRK2 levels might modulate tumor homing and outcome of tumor progression of cancer types related to inflammatory conditions (Coussens and Werb, 2002). However, whether such changes take place in the context of cancer has not been addressed.

Changes in GRK2 expression/ activity in specific tumors and functional impact

Despite the potential relationship of GRK2 with many of the processes and hallmarks of cancer discussed above, a comprehensive study of changes in its

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function or levels in specific tumoral contexts and on the role of GRK2 in tumor formation and progression has only recently started to be addressed (Fig.2).

It should be noted that GRK2 functionality and protein expression is regulated at multiple levels (Ribas *et al.*, 2007; Penela, Murga, *et al.*, 2010), so the interpretation of the absence of changes in the GRK2/ADRBK1 gene in more widely available cancer-related mRNA databases is not straightforward. Altered protein levels of GRKs that are not paralleled by corresponding changes in mRNA levels have been noted in several pathological conditions, including chronic inflammatory processes (Lombardi *et al.*, 2002), hypothyroidism, or different tumoral contexts such as in differentiated thyroid carcinoma or ovarian cancer (Métayé *et al.*, 2002; King *et al.*, 2003; Penela, Murga, *et al.*, 2010).

GRK2 protein interacts with different regulators such as Hsp90 or caveolin, and undergoes different types of post-translational modifications (reviewed in Ribas *et al.*, 2007). Kinases frequently altered in tumoral contexts such as the tyrosine kinase c-Src or MAPK/ERK1-2 can modulate GRK2 function. Tyrosine-phosphorylated GRK2 display an enhanced catalytic activity toward both GPCR and non-GPCR substrates (Sarnago *et al.*, 1999), while modification at S670 by ERK1-2 cause a switch in the repertoire of kinase substrates and interactors, allowing phosphorylation of HDAC6 (Lafarga *et al.*, 2012) while disrupting its interaction with GPCR or GIT1 (Elorza *et al.*, 2000; Penela *et al.*, 2008). Phosphorylation has also direct effects on the modulation of GRK2 stability by the proteasome pathway (Penela, 1998). Both c-Src and MAPK-dependent phosphorylation of GRK2 underlie GPCR-induced GRK2 degradation, which involves the Mdm2 E3-ubiquitin ligase (Penela *et al.*, 2001; Elorza *et al.*, 2003). Arrestins appear to play a coordinating role in recruiting kinases and/or ubiquitin

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ligases to GRK2 in the basal condition or upon activation of GPCRs, regulating GRK2 turnover via different pathways (Nogués *et al.*, 2011). Interestingly, activation of the PI3K/Akt pathway by IGF-1-triggered RTKs alters the Mdm2 phosphorylation pattern and promotes its nuclear shuttling, thus hindering Mdm2-mediated GRK2 ubiquitination, leading to boosted GRK2 protein levels (Salcedo *et al.*, 2006), suggesting that in some tumoral contexts the increase in GRK2 expression might be concurrent with a higher activity of this ligase.

GRK2 in breast tumorigenesis

Recent data from our laboratory indicate a relevant oncomodulator role for GRK2 in breast tumorigenesis (Nogués *et al.*, 2016). Elevated GRK2 protein levels are present in different breast cancer cell lines, in spontaneous mice mammary tumors, and in a significant proportion of invasive ductal carcinoma patients. The stimulation of estrogen or EGFR receptors, the Ras-HER2 and the PI3K-AKT cascades, known to be hyper-activated in luminal and in certain non-luminal types of breast cancer (Eroles *et al.*, 2012) converge in promoting enhanced GRK2 expression in transformed breast epithelial cells, likely via enhanced stimulation of the AKT pathway (Nogués *et al.*, 2016). Cancer cells where GRK2 is enhanced display genetic alterations in this pathway (*PI3KCA, PTEN*) or hyper-stimulation of receptors (EGFR, HER2, ER) able to trigger AKT stimulation, which in turn would stabilize GRK2 protein by hampering Mdm2-mediated GRK2 proteasome degradation (Salcedo *et al.*, 2006).

Augmented GRK2 levels play a driving role in the acquisition of oncogenic features by both luminal and basal breast cancer cells. GRK2 up-regulation leads to a reinforcement of EGF or heregulin-triggered mitogenic (ERK1/2) and survival (AKT) pathways, thereby enhancing growth potential under low-serum

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or normal conditions and resistance to the induction of cell death by different therapeutic agents Moreover, GRK2 up-regulation markedly favors anchorage-independent growth of luminal MCF7 or MDA-MB-231 basal cancer cells and increases their competence to trigger tumor growth *in vivo* (Nogués *et al.*, 2016). Conversely, decreasing GRK2 levels have the opposite effect in both luminal and basal breast cancer cells (Zhang *et al.*, 2014; Nogués *et al.*, 2016) and sensitize breast cancer cells to chemotherapeutic agents. Activation of the HDAC6-Pin1 axis appears to underlie the positive effects of GRK2 on oncogenic hallmarks. Increased GRK2 fosters the phosphorylation and activation of HDAC6 leading to de-acetylation of the Prolyl Isomerase Pin1, a central modulator of tumor progression, thereby enhancing its stability and functional interaction with key mitotic regulators (Fig.3). A correlation between GRK2 expression and Pin1 levels and de-acetylation status is noted in both cell models and in breast cancer patients (Nogués *et al.*, 2016).

In sum, GRK2 up-regulation emerges as a convergent feature of the stimulation of diverse pathways altered in luminal breast cancer, in parallel to that of other key proteins such as HDAC6 or Pin1, known to be over-expressed in these tumoral contexts (Lee *et al.*, 2008; Li *et al.*, 2013; Zhou and Lu, 2016). In such situations as well as in settings of increased phosphorylation of GRK2 on S670 by means of hyper-activation of ERK1/2 (often found in both luminal and basal breast cancer contexts), the switch-on of the GRK2-HDAC6-Pin1 signaling module would help to perpetuate cell proliferation and increased survival.

It is tempting to suggest that the GRK2-HDAC6-Pin1 molecular signature in breast tumorigenesis might be used as a potential target for combination therapies. Although targeting growth factors or estrogen receptors has improved

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the treatment of certain subtypes of breast cancer, the emergence of resistances is an important drawback. Partial responses to pan-HDAC inhibitors have been shown (Tate *et al.*, 2012) in GRK2-overexpressing cells, while extra GRK2 levels lessen the cytotoxic effectiveness of HDAC6 inhibitors (Nogués *et al.*, 2016). Moreover, increased GRK2 levels would favor down-modulation of wild-type p53 protein and extra activation of the pro-survival AKT route, helping to counteract the effects of cytotoxic compounds. These results are consistent with the occurrence of an inverse GRK2-p53 correlation in xenograft tumor models and in tumors of patients with breast cancer (Nogués *et al.*, 2016). Therefore, treatment of certain types of breast cancer may benefit from the combined use of GRK2, HDAC6 or Pin1 (Zhou and Lu, 2016) inhibitors.

Tumor-type specific roles of GRK2 in cancer progression

It is worth noting that changes in GRK2 levels and the overall impact of altered GRK2 expression on specific tumors might differ. Contrary to breast cancer cells, GRK2 appears to play an inhibitory role in IGF1-induced hepatocellular carcinoma cells (HCC) proliferation and migration. Overexpression of GRK2 decreases early growth response-1 (EGR1) expression, while GRK2 silencing increases EGR1 expression (Ma et al., 2016) and the MAPK pathway (Fu *et al.*, 2013). However, no information was provided in these reports on expression levels of the kinase in primary HCC tissues. Similarly, it has been reported that a GRK2-specific peptide inhibitor increased tumor mass upon xenograft transplantation of HEK293 cells (Fu *et al.*, 2013).

Regarding pancreatic cancer, GRK2/ADRBK1 gene was identified as a relevant target using a high-content screening approach (Bucholz *et al.*, 2015). RNAi-mediated knockdown of GRK2 significantly inhibited growth through induction of

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a G1/S cell cycle arrest, consistent with the notion that enhanced GRK2 fosters cancer progression. Analysis of tissue microarrays confirmed that GRK2 protein was undetectable in non-neoplastic tissues (chronic pancreatitis and normal pancreas), whereas readily detectable in 51% of pancreatic ductal adenocarcinoma samples in both epithelial cancer cells as well as subsets of infiltrating immune cells. Another group has also suggested that GRK2 is overexpressed in pancreatic cancer and might serve as potential indicator of unfavorable prognosis (Zhou *et al.*, 2016).

On the other hand, down-modulation of GRK2 has been reported in Kaposi´s sarcoma (Hu et al., 2015), leading to enhanced migration in endothelial cells, and in a subset of prostate cancers with high grades of malignancy (Prowatke *et al.*, 2007). However, another group has shown that expression of the c-terminal region of GRK2 strongly inhibited growth of prostate cancer cells *in vitro* and *in vivo,* although whether this effect was specific or due to blocking $G\beta\gamma$ signaling was not discerned (Bookout *et al.*, 2003).

Concluding remarks

In sum, the fact that GRK2 is functionally related to key hallmarks of cancer and the emerging evidence obtained in several tumors put forward this protein as an onco-modifier, able to contribute to cancer progression in different ways, depending on the specific tumor and cell types. Future research will help to characterize potentially altered GRK2 levels or function in different tumor types at different stages of progression, to better understand how oncogenic drivers and/or the tumor microenvironment promotes changes in GRK2 expression and functionality, and to uncover the molecular mechanisms by which such changes

cooperate in tumor proliferation or metastasis. In addition to transformed cells, these investigations should also include the other cell types (endothelial, fibroblasts, immune) present in the tumor microenvironment. As exemplified by the available data on breast tumorigenesis, simultaneous and opposed alterations of GRK2 in the epithelial (up-regulation) (Nogués *et al.*, 2016) and stromal (down-modulation) components of breast tumors (Rivas *et al.*, 2013) might act synergistically to promote tumor growth (Fig.4). A better knowledge of the mechanisms underlying such tumor and cell type–specific regulation of GRK2 levels and functionality and of its impact in signaling networks and cellular functions will help to gain further insight on its integrated role in cancer development, and to design future therapeutic strategies.

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Legends to Figures

Figure 1.- GRK2 modulates multiple processes related to the hallmarks of cancer. Overview of the diverse GRK2 "interactomes" (either scaffold or kinase-activity dependent) integrated in pathways controlling the hallmarks of cancer. See text for detailed discussion.

Figure 2.- Changes in GRK2 expression/ activity in specific tumors

A. Schematic representation of the tumor types where altered GRK2 expression has been reported. Experimental approaches and references are indicated. B. Available evidence supporting a role for altered GRK2 levels in tumor progression. Experimental models, the cell type targeted to alter GRK2 expression or activity, its overall impact on tumorigenesis and relevant references are indicated. See text for details.

Figure 3.- Molecular mechanisms involved in the role of GRK2 in breast tumorigenesis

Enhanced activity of different tumor-promoting cascades in breast cancer converge in promoting the upregulation of GRK2, HDAC6 and Pin1, as well as in increasing phosphorylation of GRK2 on S670. The switch-on of the GRK2-HDAC6-Pin1 signaling module would contribute to perpetuate cell proliferation and increased survival. See text for details.

Figure 4.- Physiological integration of cell-type specific GRK2 effects in breast cancer tumorigenesis

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Concurrent and opposite changes in GRK2 expression taking place in epithelial and endothelial components of breast tumors might act synergistically to promote tumor growth.

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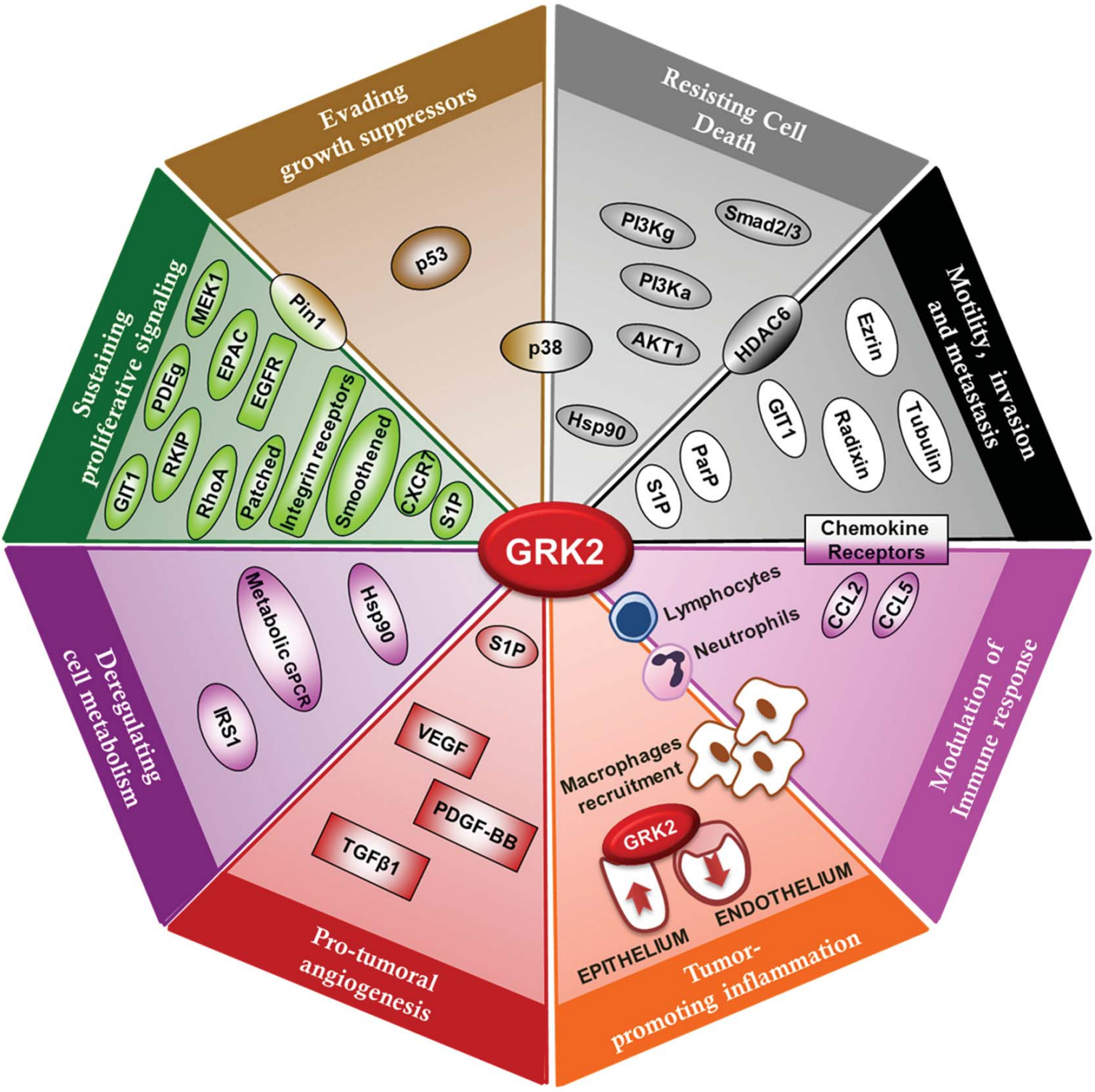
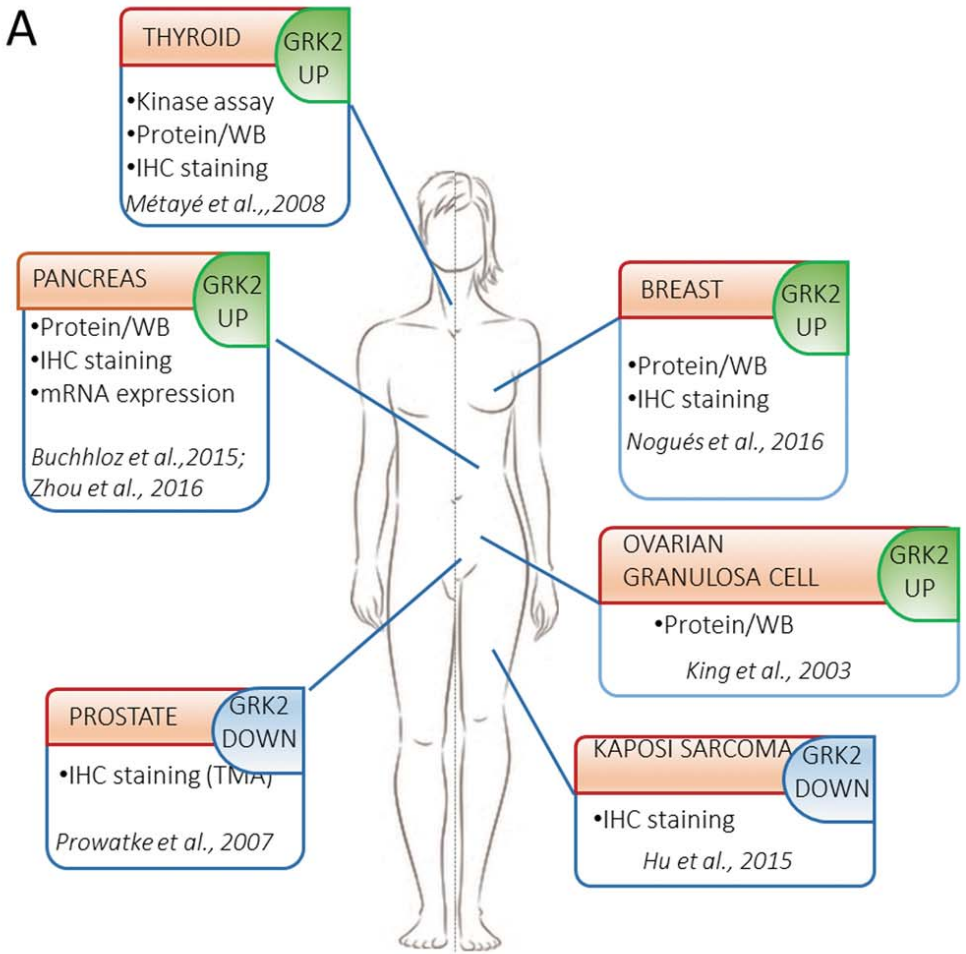


Figure 1



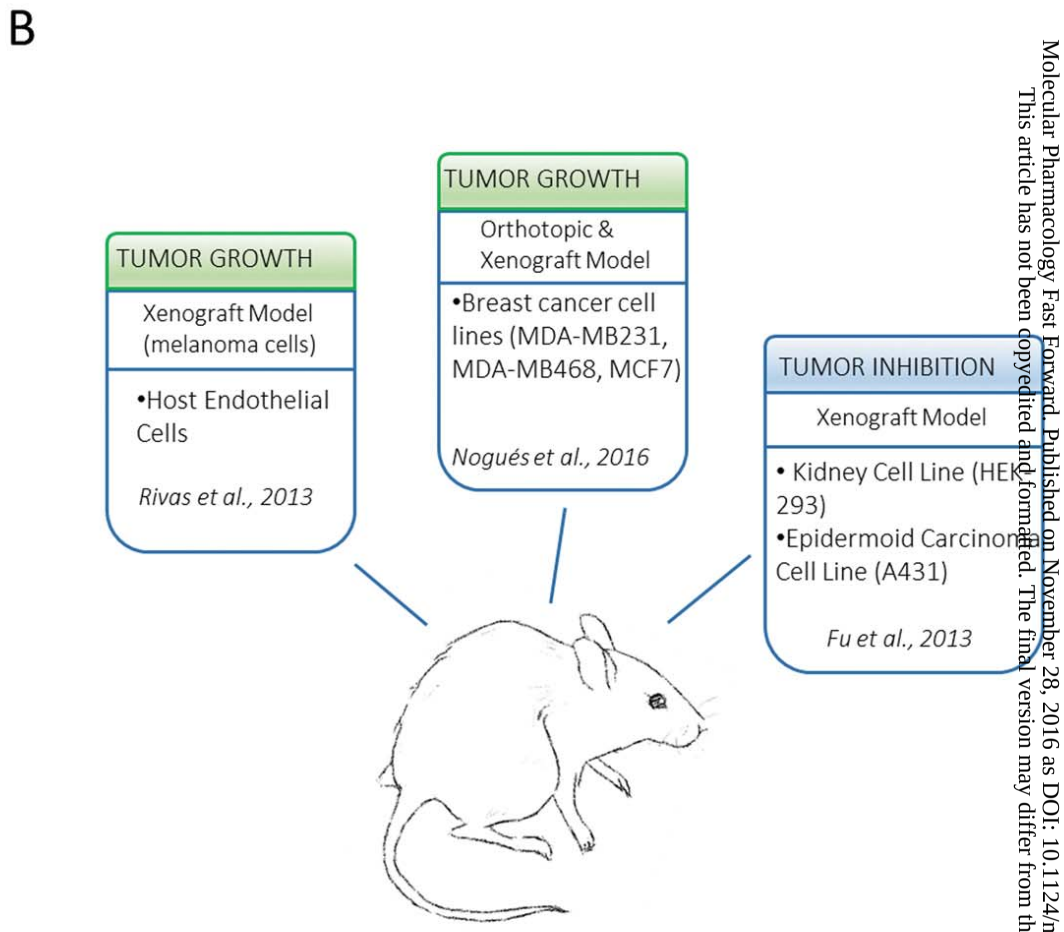


Figure 2

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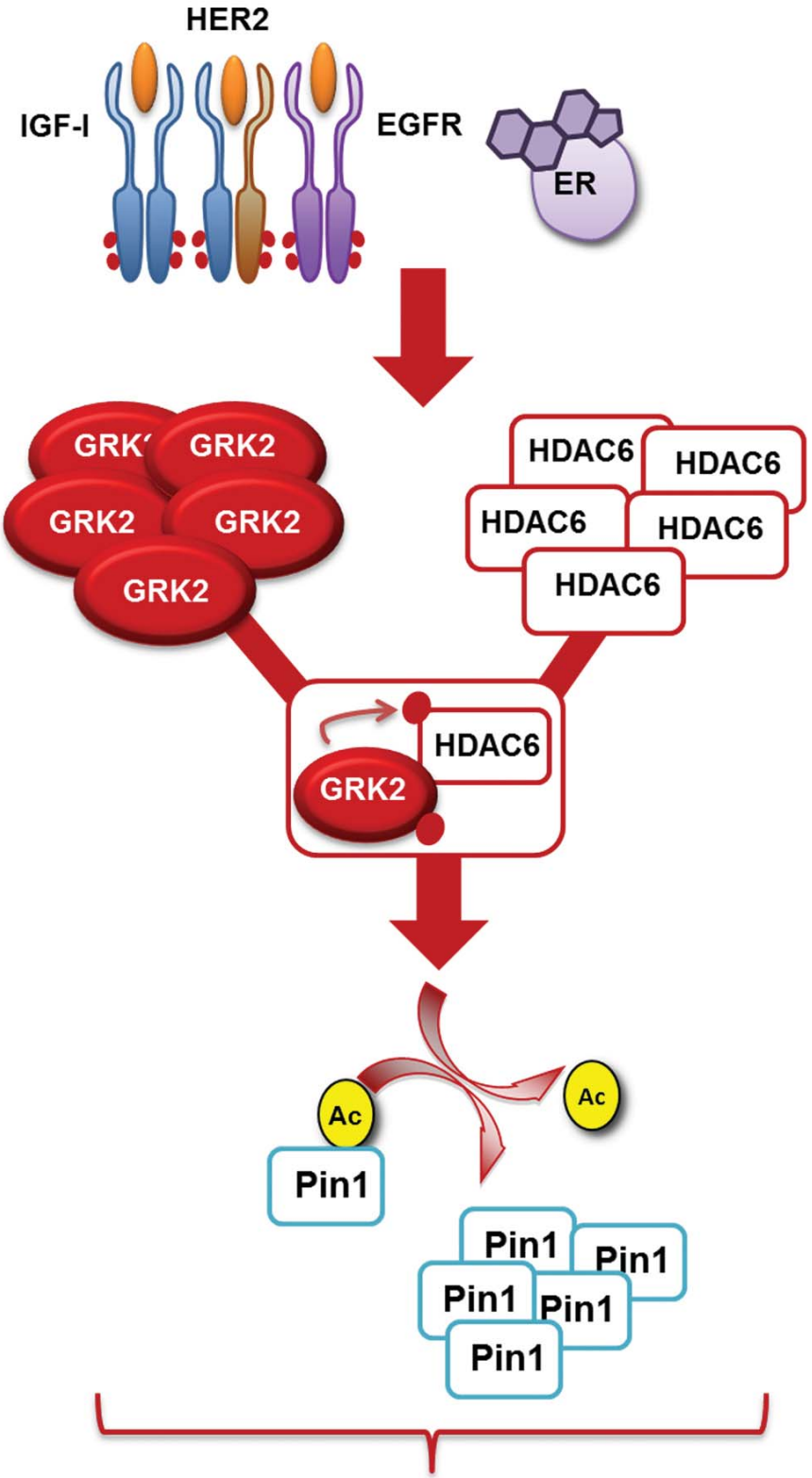

Figure 3

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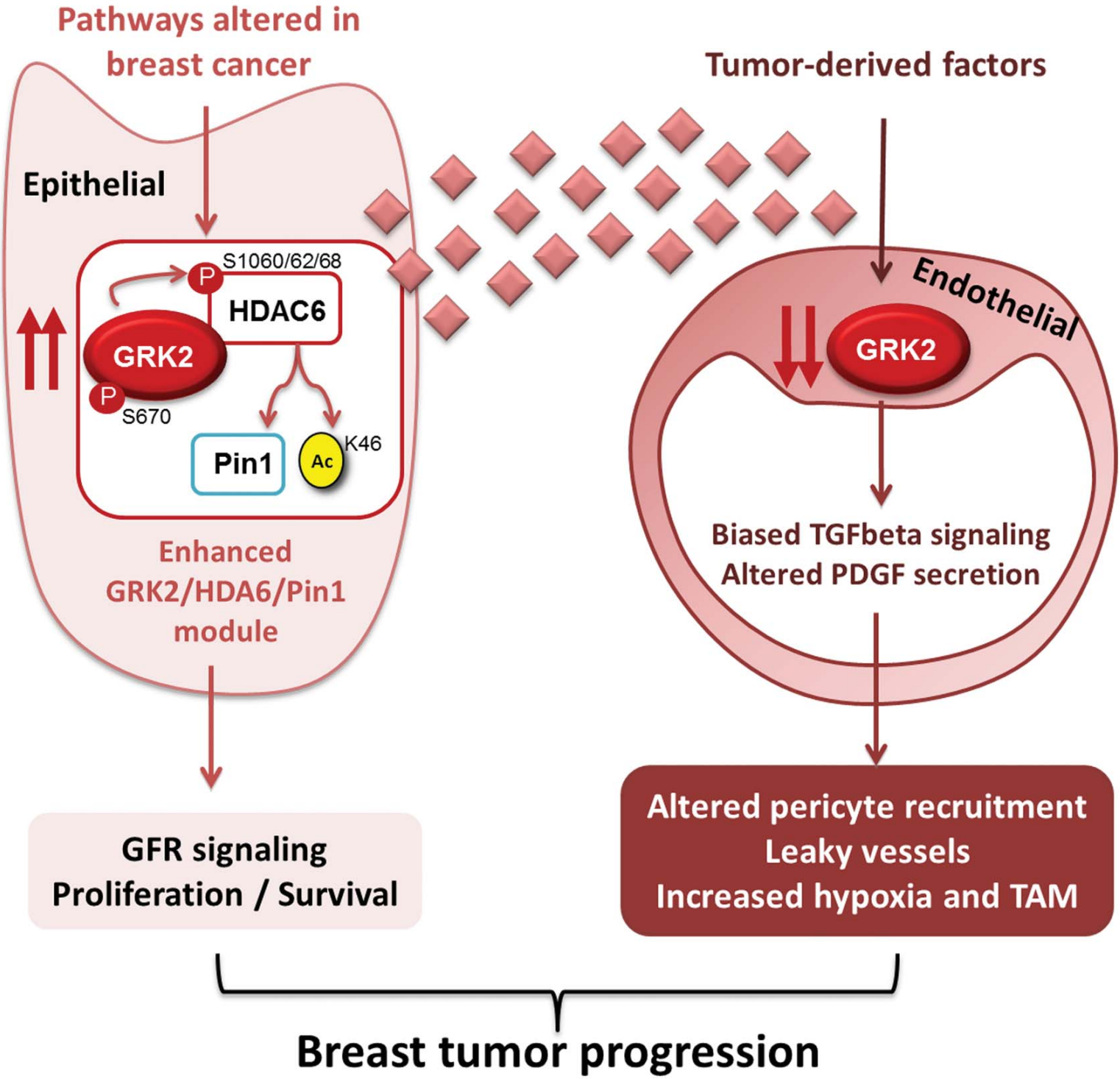

Figure 4