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Relevance of small GTPase Rac1 pathway in drug and radio-resistance mechanisms: Opportunities in cancer therapeutics

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ABSTRACT

Rac1 GTPase signaling pathway has a critical role in the regulation of a plethora of cellular functions governing cancer cell behavior. Recently, it has been shown a critical role of Rac1 in the emergence of resistance mechanisms to cancer therapy. This review describes the current knowledge regarding Rac1 pathway deregulation and its association with chemoresistance, radioresistance, resistance to targeted therapies and immune evasion. This supports the idea that interfering Rac1 signaling pathway could be an interesting approach to tackle cancer resistance.

1. Introduction

The Rho family of small GTPases consists of at least 20 members (~ 21 kDa), different from heterotrimeric G proteins (GPCRs). These proteins act as molecular switches and are critical for multiple signaling pathways that control cell behavior. The best studied Rho GTPases (Rho, Rac1 and Cdc42) are the most highly conserved Rho family members across eukaryotic species (Ridley, 2015). These proteins can cycle between an active conformation (bound to GTP) and an inactive conformation (bound to GDP) (Jaffe and Hall, 2005). Although they show intrinsic GTPase activity, this cycle is tightly regulated by other proteins such as guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs) and guanine nucleotide exchange inhibitors (GDIs). GEFs are Rho GTPase activators and catalyze the exchange of GDP for GTP, while GAPs promote the GTP hydrolysis to GDP. Additionally, GDI extract the inactive GTPase from membranes (Etienne-Manneville and Hall, 2002). It is important to note that the active GTP-bound state binds preferentially to downstream effector proteins and actively transduces signals (Ridley, 2015).

Rac1 has traditionally been described as the main regulator in actin cytoskeleton reorganization; affecting endocytosis and trafficking, cell cycle progression, adhesion and migration. Importantly, Rac1 controls lamellipodia formation and membrane ruffles after stimulation by extracellular ligands such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF) or insulin (Bustelo et al., 2007). Rac1 is also involved in transcriptional modulation of gene expression through NFkB, JNK y MAPK activation and later induction of AP-1 transcription factors involved in cell proliferation. Further, Rac1 stimulates gene expression in the nucleus through activation of c-Jun N-terminal kinase cascade (JNK) ending with c-Jun phosphorylation and activation, a central member of AP-1 complex (Coso et al., 1995). These transcription factors modulate the expression of different key regulators of cell cycle and proliferation such as cyclin D1.

Although, Rac1 has been mainly associated to regulation of cell cytoskeletal reorganization, novel activities have been described in the last decade. Of great interest, Rac1 was shown to regulate the induction of DNA damage response mechanisms in cardiomyocytes (Huelsenbeck et al., 2012). Also it was demonstrated that Rac1 is required for vascular integrity and angiogenesis having a particular role in blood vessel sprouting (Nohata et al., 2016). Furthermore, Rac1 promotes glucose uptake by regulating GLUT4 transporter during exercise (Sylow et al., 2016).

It is not surprising then, that malfunction of Rac1 GTPase-controlled signaling pathways is linked to different pathological settings, being cancer one of them. Rac1 GTPase used to be considered rarely mutated in tumors; however recent efforts in genomic sequencing have enabled the characterization of one relevant hotspot on *RAC1* gene in melanomas. *RAC1P29s* is a gain-of-function mutation, being the third most common recurrent mutation in melanoma (Krauthammer et al., 2012; Hodis et al., 2012). Further work identified the same mutation in head

Abbreviations: GEF, Guanine Exchange Factor; GAP, GTPase-activating protein; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; EGFR, epidermal growth factor receptor; Pak, p21-activated kinase; EMT, epithelial to mesenchymal transition; SCC, squamous cell carcinoma; GBM, glioblastoma multiforme

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and neck and endometrial cancers using a computational approach. In the same analysis, a second mutation harbored by *RAC1* gene is A159 V hotspot and was identified mainly in head and neck cancers (Chang et al., 2016).

RAC1P29s is considered to be a driver gene in melanoma, Rac1 mutations per se are not common, being Rac1 more frequently aberrantly activated in cancer rather than mutated (Fritz and Kaina, 2006). Rac1 GTPase activation is triggered by a variety of extracellular stimuli, ranging from growth factors (EGF, PDGF, TGF-β), G-coupled receptor agonists (SDF-1, LPA) and extracellular matrix molecules (fibronectin, collagen IV) (Buchsbaum, 2007). Therefore, alternative signaling pathways activated by different types of cell receptors converge on Rac1 GTPase activation. In this regard, changes in the status of some of these receptors are related to Rac1 hyper activation *via* GEF stimulation.

A key growth factor receptor in cancer is the EGF receptor (EGFR). EGFR is expressed at high levels in multiple cancer types and appears to promote growth in solid tumors (Nicholson et al., 2001). One important branch of EGFR signaling is through activation of GTPase Rac1, which further promotes cell proliferation and survival by activating the Rac1/PAK/RAF/MEK/ERK pathway (Arai et al., 2005) and the Rac1/c-Jun kinase cascade (Davis, 2000). In glioblastoma (GBM), for instance, EGFR gene amplification and overexpression is a striking feature. Mutant versions of this receptor have also been identified, being the most common the EGFRvIII mutant. EGFRvIII is unable to bind to its ligand, and it signals constitutively and is frequently co-overexpressed with wtEGFR (Wong et al., 1992). It has been shown that EGFRvIII promotes glioma growth and invasion through (PKA)-dependent phosphorylation of Dock180, a Rac1-GEF, stimulating Rac1 activation and glioma cell migration (Feng et al., 2014). Interestingly, another mechanism involved in EGFR-induced Rac1 activation was shown in non-small-cell lung cancer and colon cancer cells. These observations suggest that EGFR activation results in accumulation and stabilization of another Rac1 GEF: Tiam1. This effect is mediated by Akt phosphorylation, one major EGFR downstream kinase (Zhu et al., 2015). Other GEFs were also shown to be stimulated by EGFR. Vav proteins are GEFs that also are activated by tyrosine kinase receptors, such as EGFR (Pandey et al., 2000). Importantly, Vav proteins additionally exhibit GEF-independent activities during cell signaling in different scenarios (Bustelo, 2014).

Many signals activate Rac1, stimulating different effectors. The best known Rac1 effectors are P21-activated kinases (PAKs) mainly Pak1, Pak2 and Pak3, MAPK, NFkB, the adaptor protein NCK/Wave1 and the NADPH oxidase (p67 phox) (Bid et al., 2013).

One key feature in aggressive tumor cells is the acquisition of a mesenchymal phenotype by a complex process called epithelial-mesenchymal transition (EMT). Cells undergoing EMT lose their cell polarity and cell-to-cell adhesion and acquire migratory and invasive potential as well as stem-like features (Thiery et al., 2009). Rac1 protein has been associated to EMT, since this Rho GTPase is involved in cell polarity, migration, invasion and stemness (Orgaz et al., 2014). Recently it has been shown that PI3K/Akt-Rac1-JNK axis promotes EMT in gastric adenocarcinoma (Yoon et al., 2017). Moreover, PI3 K also controls migration through Rac1 modulation and EMT in squamous lung cancer (Bonelli et al., 2015; Cavazzoni et al., 2017), highlighting the role of Rac1 as key regulator of aggressiveness. Therefore, it is not surprising that Rac1 GTPase has gained increasing attention as a drug target, particularly in combination settings (Marei and Malliri, 2016).

Rac1 is involved in every single step during cancer progression (Orgaz et al., 2014) and new studies highlight the importance of Rac1 pathway activation as an adaptive advantage for cancer cells to survive and acquire resistance to current treatments. The focus of this review is to shed light on the resistance mechanisms associated to Rac1 pathway in response to different treatment options such as chemo- and radiotherapy, targeted therapy and hormone therapy in different cancer types.

2. Rac1 is involved in resistance mechanisms

Since last century, cancer treatment has become increasingly sophisticated having profound effects on disease management and patient survival. However, one of the main problems affecting cancer treatment is the emergence of resistance mechanisms to both, standard therapy and targeted-based therapies. Cancer treatments are commonly associated to different types of drug resistance: intrinsic or acquired. Patients that do not respond to therapy and are refractory are intrinsically resistant, while patients that initially are responsive usually relapse and become resistant due to acquired drug resistance. Current research efforts focus on dissecting underlying mechanisms of resistance to provide a clearer rationale to thoughtfully select patients for effective therapies and combine therapeutic agents for an improved patient outcome.

Several studies aim to find driver mechanisms as well as protein candidates associated to acquired resistance. Some of the alterations include structural changes in the drug target, resistance to apoptosis triggered by compensatory pro-survival pathways, the migratory phenotype of malignant cancer cells, the presence of cancer cells with stem-like properties within the tumors and the tumor cell-microenvironment interaction (Holohan et al., 2013). Several studies have proposed Rac1 GTPase as having a key role in many of these adaptive changes that cancer cells undergo after therapy.

2.1. Chemoresistance and Rac1

Chemotherapy is one of the most used cancer treatments, both as adjuvant and neoadjuvant modalities. Despite its wide use, the efficacy of chemotherapy is limited in some cases due to insensitivity and the development of chemoresistance. It has been shown that micro and macroevolution within the tumor cell population seems to be important in chemoresistance mechanisms (Gerlinger et al., 2014). Interestingly, the mutagenic pressure of chemotherapeutics followed by the emergence of adaptive phenotypes contributes to rapid cancer evolution and drug resistance. Ultimately, chemotherapy often fails because of the emergence of resistant cancer cells. It has been demonstrated that this emergence can be driven by the presence of chemotherapy drug gradients and motility of the cancer cells within the gradient (Wu et al., 2013). Thus, targeting of these adaptive cancer cells might have a great impact in the treatment of this disease. For example, Rac1 has been implicated in radio- and chemoresistance in head and neck squamous cell carcinoma (HNSCC). Rac1 expression in these cells is markedly enhanced after cell exposure to ionizing radiation or cisplatin treatment. Of great interest is the fact that Rac1 overall levels and nuclear expression were higher in HNSCC patients with poor treatment response and tumor relapses (Skvortsov et al., 2014). Rac1 inhibition in HNSCC cells restores anoikis, decreases cell motility and enhances cell sensitivity to standard treatment, showing a reduction in dosage of ionizing radiation or cisplatin by 1.5–3.0-fold in order to reach the same effect observed with each therapy separately (Skvortsov et al., 2014; Arnold et al., 2014). Similar results were shown using doxorubicin-resistant SCC cells, where Rac1 pharmacological inhibition reinstated doxorubicin sensitivity (Hazar-Rethinam et al., 2015). Additionally, PAK1 showed to confer cisplatin resistance in NSCLC (Chen et al., 2016). Of great interest, recently it has been reported that Rac1 inhibition could reverse 5-fluorouracil and cisplatin chemotherapy in gastric adenocarcinoma spheroids. Moreover, the *in vivo* treatment with cisplatin of Rac1 shRNA gastric adenocarcinoma cells dramatically inhibited tumor growth in a xenograft model (Yoon et al., 2017).

In leukemia cells, Rac1 has also been associated with chemoresistance. In chronic lymphocytic leukemia (CLL), Rac1 and its GEF Tiam1 are important for proliferation and chemoresistance to fludarabine, a DNA intercalating purine analogue. Interestingly, CLL cells acquired resistance towards fludarabine when cocultured with activated T cells and fibroblasts. This coculture resulted in upregulation of Tiam1

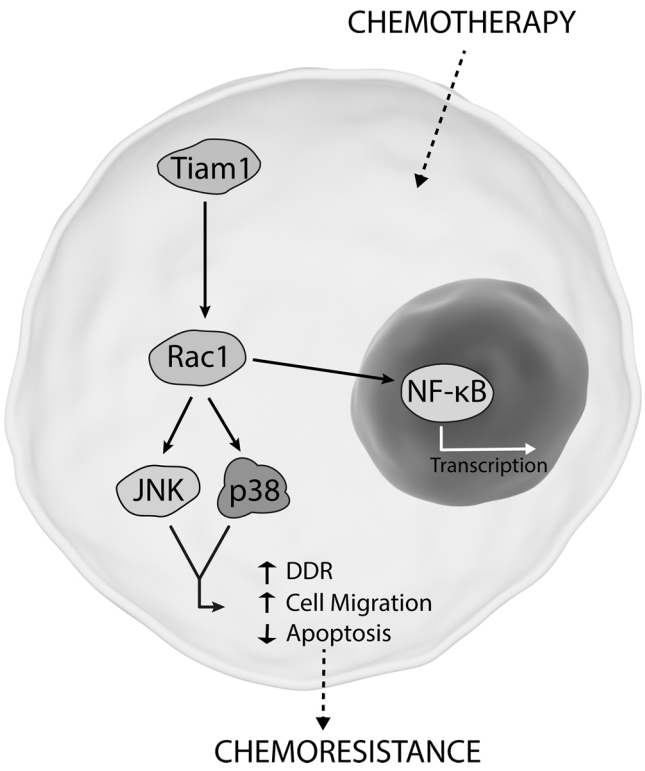

Fig. 1. *Rac1 is involved in chemoresistance mechanisms*. Rac1 is able to integrate cellular responses to genotoxic agents such as alkylating agents, by activating different stress-activated kinases like JNK, p38 kinase and different transcription factors such as NFkB. (*This scheme is a modification of the somersault1824.com free online illustrations*).

mRNA, suggesting a dynamic regulation of Rac1 function in the CLL microenvironment. However, these cells could be resensitized to fludarabine by Tiam1/Rac1 inhibition (Hofbauer et al., 2014). Inactivation of Rac1 in leukemia cells also was shown to enhance sensitivity to etoposide-induced apoptosis (Wang et al., 2013).

One of the main mechanisms by which Rac1 provides resistance to chemotherapy might be the role of this GTPase in apoptosis regulation. In this regard, studies using NIH3T3 cells showed that dominant active Rac1 expression prevents cisplatin-induced apoptosis, but the role of Rac1 GTPase in chemoresistance is not restricted to a pro-survival compensatory mechanism (33). Notably, Rac1 GTPase has an important role in genomic stability by mediating DNA damage response (DDR) pathways in response to genotoxins. Rac1 has been suggested to be connected with key regulators of genotoxic stress responses on different levels (Fritz and Henninger, 2015). Importantly, Rac1 was shown to integrate cellular responses to genotoxic agents such as alkylating agents, by activating different stress-activated kinases like SAPK/JNK, p38 kinase and different transcription factors such as NFkB and AP-1 (Fritz and Kaina, 2013) (Fig. 1).

2.2. Radioresistance and Rac1

In line with the mentioned above, radiotherapy is also an important component of cancer treatment having a direct impact on DNA damage and cell division. Although it has been shown that DNA damage response evoked by ionizing radiation is not regulated by Rac1 GTPase in a liver fibrosis model (Bopp et al., 2013), Rac1 has been associated to radioresistance in other cases (Hein et al., 2016; Yan et al., 2014; Zhou et al., 2016; Espinha et al., 2015). One possible link between radioresistance and Rac1 might be the crucial regulation of EMT by Rac1 GTPase (Fang et al., 2017; Gulhati et al., 2011). It has been shown that EMT determines therapy response and tumor progression. On the other hand, radiation-induced EMT was associated with enhanced cancer cell migration and invasion (Zhou et al., 2011; Jung et al., 2007). But the exact mechanism still needs to be addressed.

Rac1 appears to also have important nuclear activities in addition to the well documented regulated cytosolic events. Using a FRET biosensor it was demonstrated that Rac1 distributes itself between the nucleus and cytoplasm upon induction of DNA damage caused by irradiation. Interestingly, active Rac1 accumulates in the nucleus and its signaling pathway has a direct role in the regulation of nucleocytoplasmic transport during DNA repair (Hinde et al., 2014). Nuclear translocation of PAK1 can also be stimulated by ionizing radiation (IR) and nuclear PAK1 associates with chromatin causing direct alterations in gene expression, having a profound influence on the p53 pathway (Motwani et al., 2013) (Fig. 2). Supporting evidence also shows that IR activates the G2/M checkpoint in breast cancer MCF7 cells and this activation is Rac1 dependent (Yan et al., 2012). Using HeLa cells, gamma and UV radiation lead to augmented levels of active Rac1. DNA damage was increased when these cells had reduced Rac1-GTP levels, dramatically impairing the recovery of these cells after radiation exposure (Espinha et al., 2015).

Evidence shows that Rac1 signaling is essential for the survival of cancer cells subjected to radiation. Rac1 is implicated in radioresistance in different cancer cell types such as breast cancer (Hein et al., 2016; Yan et al., 2012), GBM (Zhou et al., 2016; Yoon et al., 2011), pancreatic cancer (Yan et al., 2014), cervical cancer (Espinha et al., 2015) among others.

2.3. Resistance to targeted therapy and Rac1

Although traditional cytotoxic chemotherapy and radiotherapy remain the treatment of choice for many types of cancer, targeted therapies have become a central component of treatment. Targeted-therapeutic approaches are very effective in many cancer types but there is growing evidence that any given agent is likely to fail because of acquired resistance. The establishment of specific molecular mechanisms of resistance is very challenging due to the plethora of

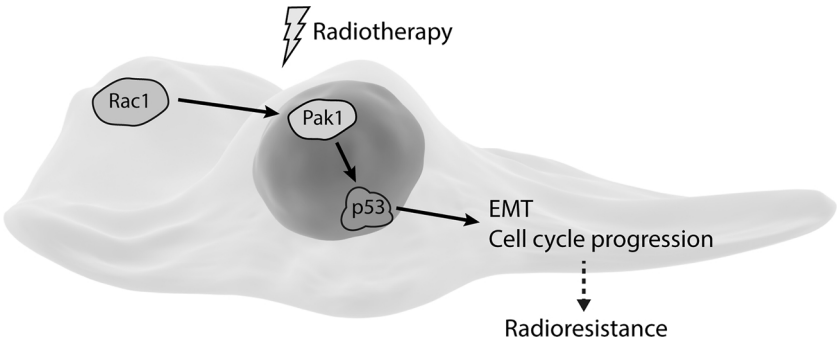

Fig 2. *Rac1 has been associated to radioresistance*. The translocation to the nucleus of Pak1 (a Rac1 downstream effector) influences p53 activity resulting in radioresistance. (*This scheme is a modification of the somersault1824.com free online illustrations*).

mutations present in the cancer cells after treatment, their interaction with the surrounding microenvironment and the tumor heterogeneity (Ramos and Bentires-Alj, 2015). All these components usually prevent long-term efficacy of any targeted mono-therapy. These setbacks put an emphasis on discovering the means to increase the efficacy of targeted therapies and to overcome resistance.

Receptor tyrosine kinases (RTKs) are cell surface receptors with a well-known role in aberrant mitogenic signaling in cancer cells, such as EGFR. Therefore, it is not surprising that several drugs have been developed and approved for treating cancers caused by activated RTKs (Lemmon and Schlessinger, 2010). Convincing evidence suggests that Rac1 serves as a downstream effector for multiple RTKs (Etienne-Manneville and Hall, 2002) and Rac1activation appears to be a compensatory mechanism in response to RTKs inhibition.

In GBM, 40% to 50% of the tumors overexpress HER1/EGFR, showing similar values in NSCLC and pancreatic carcinoma (Karpel-Massler et al., 2009). Despite the high hopes raised by the use of HER1/EGFR–targeted agents such as erlotinib for glioma treatment, this therapy failed in the clinic. Based on analysis of gene expression, Rac1 has been proposed as a candidate gene for conferring GBM resistance (Halatsch et al., 2009) and concomitant inhibition of HER1/EGFR and RAC1 results in a synergistic effect and this is associated with a downregulation of PI3K/AKT and MAPK pathways (Karpel-Massler et al., 2013; Karpel-Massler et al., 2017) (Fig. 3). In line with this idea, Rac1 inhibition was also effective in NSCLC cells resistant to gefitinib, another EGFR inhibitor (Kaneto et al., 2014).

HER2 inhibition by the monoclonal antibody trastuzumab accrues significant clinical benefit in adjuvant settings in HER + breast cancer. Rac1 activation has shown to be critical in trastuzumab resistance caused by PTEN deletion or IGF-IR overexpression (Zhao et al., 2011). Additionally, Rac1 inhibition also restores trastuzumab-mediated endocytic regulation of HER2 (Dokmanovic et al., 2009).

Other studies also associated Rac1 with antiangiogenic targeted-agents. It has been shown that Rac1 inhibition increases sensitivity of prostate tumors to bevacizumab, a monoclonal antibody targeting VEGF, and it seems that the combination of the VEGF/VEGFR-targeted therapies with Rac1 inhibition could translate into the improvement of the therapeutic response (Goel Hira et al., 2016).

Finally, RAC1 P29S hotspot mutant confers resistance to B-RAF inhibitors (vemurafenib and dabrafenib), and silencing of this mutant protein increases sensitivity to these inhibitors (Watson et al., 2014). In line with these findings, recently it has been shown that activated PAK1 confers resistance to B-RAF inhibitors, while PAK1 suppression had a sensitizing effect (Babagana et al., 2017). Altogether, this shows that Rac1 signaling pathway might have a critical role in resistance to B-RAF inhibitors.

2.4. Resistance to anti-hormonal therapy and Rac1

One particular type of targeted therapy changed the cancer therapeutic paradigm nearly 50 years ago: endocrine therapy (Jordan, 2008). This kind of therapy exploits the dependence of certain types of tumors towards hormone stimulation. This is the case of breast cancer, where endocrine therapy decreases the growth of a particular group of tumors (estrogen-sensitive) by blocking the production of estrogen or by interfering with the pro-proliferative effects of estrogen. More than 70% of breast cancer cases are estrogen-sensitive tumors and there are many types of endocrine therapies to treat them. Aromatase inhibitors, like anastrozole and letrozole, block the enzyme that produces estrogen in the ovaries and in other tissues. On the other hand, drugs known as selective estrogen receptor modulators (SERMs) like tamoxifen (Tam) interfere estrogen binding to estrogen receptor (ER) blocking growth of breast cancer cells. The clinical use of Tam has significantly improved survival in breast cancer patients. However, despite its success and very low side effects, development of resistance mechanisms is still a major problem (Musgrove and Sutherland, 2009).

De novo resistance mechanisms are described for a subgroup of patients, where the lack of expression of ER or mutations on cytochrome P450 2D6 (CYP2D6), that converts Tam to its active metabolite are found. However, the most common type of Tam resistance is acquired. The mechanisms associated with this kind of resistance include changes in the expression and activation of cell cycle and cell survival proteins, as well as the over activation of signaling pathways that allow tumor cells to escape to the inhibitory effects of Tam and proliferate. Overexpression of EGFR, human Erb B2 (HER2), and insulin-like growth factor I (IGF-I) receptors activate downstream proteins like AKT and MAPK which in turn modulate the normal functioning of ER signaling, resulting in endocrine resistance mechanisms (Morrison et al., 2014). Moreover, the PI3 K/Akt/mTOR pathway has been implicated in the development of endocrine resistance. AKT stimulates ER signaling pathway in an estrogen-independent manner and several clinical trials have shown that the mTOR inhibitor everolimus is able to overcome endocrine resistance mechanisms (Paplomata and O'Regan, 2014; Baselga et al., 2012; Beaver and Park, 2012; Bachelot et al., 2012).

Breast and prostate cancer have several features in common. The majority of breast and prostate cancer cases are adenocarcinomas

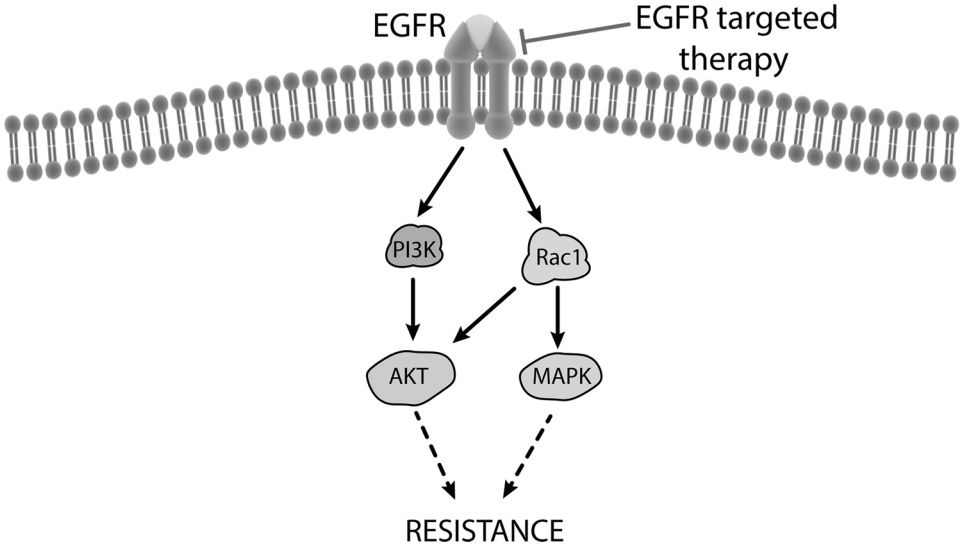

Fig. 3. *Rac1 activation confers resistance to EGFR-targeted therapy*. One possible compensation mechanism is the activation of Akt, resulting in enhanced resistance to anti-EGFR therapy. MAPK pathway may also be involved. (*This scheme is a modification of the somersault1824.com free online illustrations*).

arising in sexual organs. Therefore, both are strongly influenced by sexual hormones like estrogen and androgen. In this context, endocrine therapies are also effective for androgen receptor (AR) positive tumors. Flutamide and bicalutamide are both pure antiandrogen agents that inhibit the androgen binding to AR and its subsequent translocation to nucleus. Treatment for prostate cancer patients involves androgen ablation by surgery, endocrine therapies or a combination of both. However, relapses are very common. Several mechanisms have been described to explain prostate cancer resistance mechanisms: amplification of AR gene, an increase in androgen circulating levels, AR mutations and activation of different signaling pathways like MAPKs or AKT that overcome AR inhibition (Rau et al., 2005; Risbridger et al., 2010; Petkovic et al., 2012).

In summary, the activation of many ER- and AR-independent pathways is directly linked to acquired resistance to endocrine therapies in breast and prostate cancer. It is interesting to note that Rac1 is a convergent node in both EGFR and PI3K/AKT/mTOR pathways, being an interesting target in these settings. In line with this evidence, Rac1 has been proposed to be an important component in the development of acquired endocrine resistance in both types of cancer. One of the first results that linked Rac1 to endocrine resistance was the development of different breast cancer models that overexpressed a constitutively active form of Rac1 or the GDP exchange factor (GEF) AND-34/BCAR3. The overexpression of either of these proteins induces antiestrogen resistance (Cai et al., 2003). Moreover, the inhibition of Rac1 displayed anti-proliferative effects in Tam resistant breast cancer cells (Rosenblatt et al., 2011). Rac1 is upregulated in PC-3 prostate cancer cells and treatment of these cells with the Rac1 inhibitor NSC23766 affected its proliferation and invasion (Gao et al., 2004). Moreover, 22Rv1 prostate cancer cells proliferation, migration and tumor growth was suppressed by AZA1, a Rac1/Cdc42 inhibitor developed by virtual screening and based on the NSC23766-Rac1 interaction (Zins et al., 2013). Other members of the Rac1 pathway have also been involved in endocrine resistance. The Rac1 GEF Vav3 was identified as a critical mediator of endocrine therapy resistance in breast in a genome-wide association study (Aguilar et al., 2014). On the other hand, the main downstream effector of Rac1, PAK1, was also involved in the development of endocrine resistance. It has been shown that PAK1 could phosphorylate ER at the N-terminal residue Ser305 and increased the expression of Cyclin D1 (Balasenthil et al., 2004; Tao et al., 2011). The correlation of phosphorylation of this ER specific residue and nuclear localization of PAK1 is one of the main mechanisms associated with Tam resistance in patients (Holm et al., 2009; Holm et al., 2006; Bostner et al., 2010). Taking into account these preclinical and clinical results, our group developed a breast cancer cell model with Rac1 upregulated activity to explore several Rac1-dependent mechanisms in acquired endocrine resistance. The over activation of Rac1 pathway in this breast cancer model not only displayed an estrogen-independent and Tam-resistant phenotype but also showed an increase in PAK1 activation and nuclear localization with the subsequent increase in ER Ser305 phosphorylation. The treatment of these cells with a Rac1 inhibitor reverted these endocrine resistance mechanisms, showing once more that inhibition of Rac1 pathway may provide benefits to endocrine resistance therapies (Gonzalez et al., 2017) (Fig. 4).

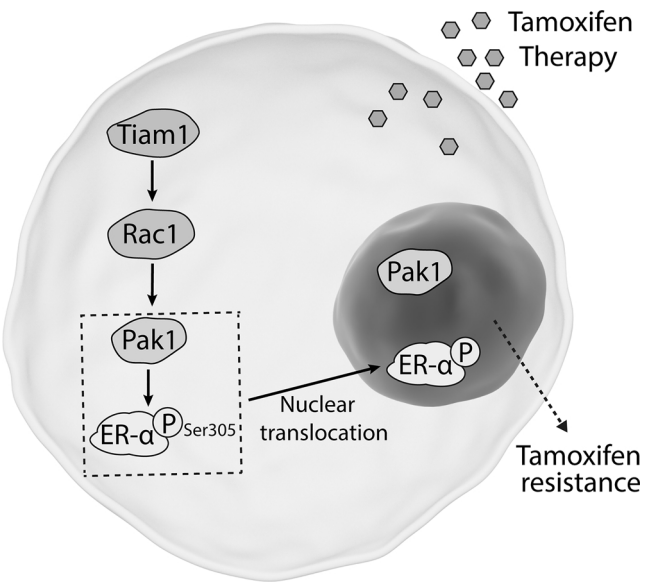

Fig 4. *Rac1 is involved in anti-hormonal therapy resistance.* In breast cancer cells, Rac1 inhibition reduced Pak1 nuclear translocation and reduced ER phosphorylation in Ser305 resulting in Tamoxifen sensibility restoration. (*This scheme is a modification of the somersault1824.com free online illustrations*).

2.5. Immune evasion and Rac1

The process of cancer immunoediting comprises three distinct stages: elimination, equilibrium and escape. Host immune factors are able to destroy tumors early before clinical manifestations, but residual tumor cells can survive and keep in quiescence. Later on, during the immune escape stage, tumor cells gain the ability to evade immune attack and thus progress to cancer. In recent years, knowledge about immune evasion has yielded the development of new strategies of cancer immunotherapy, even in indications such as non-small cell lung cancer, in which oncologists were skeptical of the value of immunomodulatory agents (Rolfo et al., 2014). In tumors where Rac1 is implicated in aggressiveness and resistance to therapy, such as glioblastoma and breast cancer, immune evasion has been recognized as a promising hallmark. Additionally to the immune-privileged state of the brain, glioblastoma cells can create an immunosuppressive microenvironment to escape immune surveillance(Razavi et al., 2016). Likewise, breast cancer cells can escape from host immune response through various strategies, including the modification of cell-surface antigens and alterations of their surrounding tissue environment (Wang et al., 2017).

Immune checkpoint-inhibitors may help to overcome immunosuppressive tumor microenvironment. Monoclonal antibodies that target the inhibitory ligand PD-L1 or its receptor PD-1 have shown surprising therapeutic efficacy in advanced melanoma. Recently, Vu and coworkers (Vu et al., 2015) demonstrated that PD-L1 expression was significantly increased in samples from melanoma patients having Rac1 P29S mutations. This finding suggests Rac1-controlled signaling pathways are able to promote suppression of antitumor immune response, and authors propose that Rac1 P29S mutations may derive greater benefit from anti-PD-L1 therapy (Vu et al., 2015). We can also speculate that specific inhibition of Rac1 in tumor cells could be a promising approach to hamper immune evasion mechanisms. However, it is important to note that targeting Rac1 in normal T lymphocytes may suppress certain immune responses. Immunosuppressive signals of chronic lymphocytic leukemia has been associated to impaired Rho-GTPase activation in T-cells (Ramsay et al., 2012). A recent study demonstrated that a 6-thioguanosine triphosphate targets Rac1 to form a biologically inactive adduct and thus the Rho GTPase activator GEF cannot exchange GDP for GTP (Shin et al., 2016). This compound is converted in T-cells from 6-thiopurine prodrugs (*e.g.* 6-thioguanine and azathioprine), that are widely used to treat autoimmune disorders, as well as cancer. Such mechanism seems to be relevant in the therapeutic action in autoimmune disease, but also may be linked to potential adverse immunosuppressive effects during cancer chemotherapy.

3. Rac1 pathway as a therapeutic target

In the last years Rac1 pathway has been pointed out as a promising molecular target due to its critical role in tumor progression as several reviews have described in detail (Fritz and Kaina, 2006; Bid et al., 2013;

Marei and Malliri, 2016; Mardilovich et al., 2012).

One of the main challenges to inhibit Rac1 is that it is not a classical druggable target like other ATPses, since the low picomolar binding affinity of small GTPases for GTP and millimolar cellular concentrations of GTP makes it implausible to develop GTP-competitive inhibitors (Vigil et al., 2010). Alternative strategies were studied to restore normal Rac1 signaling in cancer cells. One well-documented approach is the inhibition of cell membrane anchoring of Rac1 by affecting C-terminal isoprenylation. HMG-CoA-reductase inhibitors (statins) might have a great potential in particular clinical settings (Fritz and Kaina, 2013), but these inhibitors are not specific, since they do not only affect Rac1 GTPase (Wang et al., 2008).

A different strategy is to inhibit Rac1 interaction with its activators or effectors. Protein-protein interactions are viewed as challenging targets and in some cases are considered to be virtually "undruggable". This notion changed with the realization that the interaction driving the affinity of proteins is not distributed evenly across their surfaces. Rather, certain residues or regions (hot spots) are responsible for the binding (Scott et al., 2016). Based on this idea, the identification of the specific residues involved in the interaction between Rac1 and its interacting proteins makes possible to design inhibitors to mask those residues. This strategy was used to develop the classic Rac1 inhibitor NSC23766 (Gao et al., 2004) and its analogs such as E-Hop-016 (Ferri et al., 2009; Dharmawardhane et al., 2013), as well as ZINC69391 family of compounds (Cardama et al., 2014a; Cardama et al., 2014b). ZIN69391 was identified using a docking-based virtual library screening approach, where more than 200.000 compounds were screened from the ZINC publicly available database (Irwin and Shoichet, 2005). This compound was able to inhibit Rac1-Tiam1 and Rac1-DOCK180 interaction *in vitro*. This resulted in reduced Rac1 activation levels and inhibition of PAK activation. Using ZINC69391 as a parental structure, 1A116 was rationally designed to increase potency but to maintain selectivity. 1A116 showed to inhibit Rac1-P-Rex1 interaction. Interestingly, these compounds were tested in breast cancer (Cardama et al., 2014a), GBM (Cardama et al., 2014b) and leukemia (Cabrera et al., 2017) and they showed to have a profound effect on Rac1-regulated cancer cell events controlling transformed cell behavior. Of great interest, ZINC69391 family of compounds showed to trigger apoptotic cell death programs in glioblastoma and leukemia cells. Importantly, they also showed to have a profound impact on cell migration and invasion *in vitro*. These results were also shown *in vivo* using an experimental metastasis model, where ZINC69391 and 1A116 inhibitors were able to reduce 60% lung colonization by breast cancer cells compared to the control group (Cardama et al., 2014a). These results evidence a great potential for the use of 1A116 in cancer treatment and represents a promising Rac1 selective inhibitor with clinical applicability. More preclinical studies are warranted to establish particular therapeutic windows associated with enhanced therapeutic benefits.

A similar approach for targeting protein-protein interaction was taken to develop Rac1-effector inhibitors such as Phox-I1 compound (Bosco et al., 2012). The other classical Rac1 inhibitor is EHT 1864 (Shutes et al., 2007). In this case, this compound is able to displace the guanine nucleotide of Rac1. In addition to these inhibitors targeting particularly Rac1 or Rac1 activation, inhibitors of Rac1 GEFs (Blangy et al., 2006) and Rac1 effectors were also developed, such as Pak1 inhibitors (Murray et al., 2010; Nheu et al., 2002; Porchia et al., 2007).

Targeting Rac1 pathway has become an interesting strategy to control cellular events associated with the malignant phenotype and tumor progression, such as invasion and resistance to apoptosis. Preclinical data show that targeting Rac1 is feasible and effective *in vivo*, but further studies are warranted to assess the specific impact of Rac1 inhibition in clinical settings, to define the possible therapeutic windows and schemes and to establish which group of patients would benefit from this therapeutic approach. In line with this idea, the combinational potential of Rac1 inhibitors with established therapeutic agents is growing and more therapeutic opportunities are emerging.

4. Final remarks

Although recent advances in cancer therapeutics have resulted in significant prolonged survival for patients with different cancer types, more treatment options still need to be explored. Drug resistance to chemotherapy as well as targeted therapies has emerged as the real challenge in cancer therapeutics. Basic and translational research still need to address the mechanisms underlying resistance to therapeutic agents.

The identification of relevant nodes in the signaling network that orchestrate tumor cell behavior is necessary to design efficient therapeutic combination schedules. Targeting of the most critical parts of a network can have dramatic effects (Westin, 2015), and this rationale should be used for coordinated specific inhibition of the key signaling nodes. The combination of different therapeutic agents may be able to cause this complex signaling network to collapse and to have a dramatic result on tumor cell fate and a beneficial effect on patient outcome.

Rac1 has a well-documented and long-recognized role in tumor progression and metastasis but initial evidence describes Rac1 as central player in diverse compensatory mechanisms in response to therapy, placing this protein in the spotlight as a potential candidate for cancer treatment and overcoming therapy resistance. Rac1 inhibitors are already being tested in the preclinical development phase and show promising results.

Establishing the potential cancer patients that could clinically benefit from the combination of Rac1 inhibitors with established therapies would become a priority. In this regard, we have reported the potential use of Rac1 inhibitor to restore hormonotherapy sensitivity in breast cancer and this could be also be evaluated in prostate cancer. Another interesting combinational setting could be glioblastoma, where Rac1 inhibition could improve the outcome of patients treated with temozolomide and radiotherapy. Targeted-therapies, such as EGFR or HER2 inhibitors and BRAF inhibitors, could be potentiated with Rac1 inhibitors in breast cancer, melanoma and lung cancer. Since Rac1 has shown to be also involved in immune evasion, combination of Rac1 inhibitors with pembrolizumab or nivolumab could have a promising effect in melanoma treatment. Although further studies are needed, collectively the results obtained so far, open up a new opportunity to control cancer treatment resistance.

Conflict of interest

GAC, DFA and PLM served in a consultant/advisory role for Chemo-Romikin S.A. All other authors have no conflict of interests to declare.

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References

Aguilar, H., Urruticoechea, A., Halonen, P., Kiyotani, K., Mushiroda, T., Barril, X., Serra-Musach, J., Islam, A., Caizzi, L., Di Croce, L., Nevedomskaya, E., et al., 2014. Va3 mediates resistance to breast cancer endocrine therapy. Breast Cancer Res. 16 (3), R53.

Arai, A., Jin, A., Yan, W., Mizuchi, D., Yamamoto, K., Nanki, T., Miura, O., 2005. Sdf-1 synergistically enhances il-3-induced activation of the raf-1/mek/erk signaling

pathway through activation of rac and its effector pak kinases to promote hematopoiesis and chemotaxis. Cell Signal. 17 (4), 497–506.
Arnold, C.R., Abdelmoez, A., Thurner, G., Debbage, P., Lukas, P., Skvortsov, S., Skvortsova, I.-I., 2014. Rac1 as a multifunctional therapeutic target to prevent and combat cancer metastasis. Oncoscience 1 (8), 513–521.
Babagana, M., Johnson, S., Slabodkin, H., Bshara, W., Morrison, C., Kandel, E.S., 2017. P21-activated kinase 1 regulates resistance to BRAF inhibition in human cancer cells. Mol. Carcinog. 56 (5), 1515–1525.
Bachelot, T., Bourgier, C., Cropet, C., Ray-Coquard, I., Ferrero, J.M., Freyer, G., Abadie-Lacourtoisie, S., Eymard, J.C., Debled, M., Spaeth, D., Legouffe, E., et al., 2012. Randomized phase ii trial of everolimus in combination with tamoxifen in patients with hormone receptor-positive, human epidermal growth factor receptor 2-negative metastatic breast cancer with prior exposure to aromatase inhibitors: a gineco study. J. Clin. Oncol. 30 (22), 2718–2724.
Balasenthil, S., Barnes, C.J., Rayala, S.K., Kumar, R., 2004. Estrogen receptor activation at serine 305 is sufficient to upregulate cyclin d1 in breast cancer cells. FEBS Lett. 567 (2–3), 243–247.
Baselga, J., Campone, M., Piccart, M., Burris 3rd, H.A., Rugo, H.S., Sahmoud, T., Noguchi, S., Gnant, M., Pritchard, K.I., Lebrun, F., Beck, J.T., et al, 2012. Everolimus in postmenopausal hormone-receptor-positive advanced breast cancer. N. Engl. J. Med. 366 (6), 520–529.
Beaver, J.A., Park, B.H., 2012. The bolero-2 trial: the addition of everolimus to exemestane in the treatment of postmenopausal hormone receptor-positive advanced breast cancer. Future Oncol. (Lond. Engl.) 8 (6), 651–657.
Bid, H.K., Roberts, R.D., Manchanda, P.K., Houghton, P.J., 2013. Rac1: an emerging therapeutic option for targeting cancer angiogenesis and metastasis. Mol. Cancer Ther. 12 (10), 1925–1934.
Blangy, A., Bouquier, N., Gauthier-Rouvière, C., Schmidt, S., Debant, A., Leonetti, J.-P., Fort, P., 2006. Identification of trio-gefd1 chemical inhibitors using the yeast exchange assay. Biol. Cell 98 (9), 511–522.
Bonelli, M.A., Cavazzoni, A., Saccani, F., Alfieri, R.R., Quaini, F., La Monica, S., Galetti, M., Cretella, D., Caffarra, C., Madeddu, D., Frati, C., et al., 2015. Inhibition of pi3k pathway reduces invasiveness and epithelial-to-mesenchymal transition in squamous lung cancer cell lines harboring pik3ca gene alterations. Mol. Cancer Ther. 14 (8), 1916–1927.
Bopp, A., Wartlick, F., Henninger, C., Kaina, B., Fritz, G., 2013. Rac1 modulates acute and subacute genotoxin-induced hepatic stress responses, fibrosis and liver aging. Cell. Death. Dis. e558.
Bosco, E.E., Kumar, S., Marchioni, F., Biesiada, J., Kordos, M., Szczur, K., Meller, J., Seibel, W., Mizrahi, A., Pick, E., Filippi, M.-D., et al., 2012. Rational design of small molecule inhibitors targeting the rac gtpase – p67(phox) signaling axis in inflammation. Chem. Biol. 19 (2), 228–242.
Bostner, J., Skoog, L., Fornander, T., Nordenskjold, B., Stal, O., 2010. Estrogen receptor-alpha phosphorylation at serine 305, nuclear p21-activated kinase 1 expression, and response to tamoxifen in postmenopausal breast cancer. Clin. Cancer Res. 16 (5), 1624–1633.
Buchsbaum, R.J., 2007. Rho activation at a glance. J. Cell Sci. 120 (7), 1149–1152.
Bustelo, X.R., Sauzeau, V., Berenjeno, I.M., 2007. Gtp-binding proteins of the rho/rac family: regulation, effectors and functions in vivo. Bioessays 29 (4), 356–370.
Bustelo, X.R., 2014. Vav family exchange factors: an integrated regulatory and functional view. Small GTPases 5 (2), e973757.
Cai, D., Iyer, A., Felekkis, K.N., Near, R.I., Luo, Z., Chernoff, J., Albanese, C., Pestell, R.G., Lerner, A., 2003. And-34/bcar3, a gdp exchange factor whose overexpression confers antiestrogen resistance, activates rac, pak1, and the cyclin d1 promoter. Cancer Res. 63 (20), 6802–6808.
Cardama, G.A., Comin, M.J., Hornos, L., Gonzalez, N., Defelipe, L., Turjanski, A.G., Alonso, D.F., Gomez, D.E., Menna, P.L., 2014a. Preclinical development of novel rac1-gef signaling inhibitors using a rational design approach in highly aggressive breast cancer cell lines. Anticancer Agents Med. Chem. 14 (6), 840–851.
Cardama, G.A., Gonzalez, N., Ciarlantini, M., Gandolfi Donadio, L., Comin, M.J., Alonso, D.F., Menna, P.L., Gomez, D.E., 2014b. Proapoptotic and antiinvasive activity of rac1 small molecule inhibitors on malignant glioma cells. Onco Targets Ther. 7, 2021–2033.
Cabrera, M., Echeverria, E., Lenicov, F.R., Cardama, G., Gonzalez, N., Davio, C., Fernández, N., Menna, P.L., 2017. Pharmacological Rac1 inhibitors with selective apoptotic activity in human acute leukemic cell lines. Oncotarget 8 (58), 98509–98523.
Cavazzoni, A., La Monica, S., Alfieri, R., Ravelli, A., Van Der Steen, N., Sciarrillo, R., Madeddu, D., Lagrasta, C.A.M., Quaini, F., Bonelli, M., Fumarola, C., et al., 2017. Enhanced efficacy of akt and fak kinase combined inhibition in squamous cell lung carcinomas with stable reduction in pten. Oncotarget 8 (32), 53068–53083.
Chang, M.T., Asthana, S., Gao, S.P., Lee, B.H., Chapman, J.S., Kandoth, C., Gao, J., Socci, N.D., Solit, D.B., Olshen, A.B., Schultz, N., et al., 2016. Identifying recurrent mutations in cancer reveals widespread lineage diversity and mutational specificity. Nat. Biotechnol. 34 (2), 155–163.
Chen, M.J., Wu, D.W., Wang, Y.C., Chen, C.Y., Lee, H., 2016. Pak1 confers chemoresistance and poor outcome in non-small cell lung cancer via beta-catenin-mediated stemness. Sci. Rep. 34933.
Coso, O.A., Chiariello, M., Yu, J.C., Teramoto, H., Crespo, P., Xu, N., Miki, T., Gutkind, J.S., 1995. The small gtp-binding proteins rac1 and cdc42 regulate the activity of the jnk/sapk signaling pathway. Cell 81 (7), 1137–1146.
Davis, R.J., 2000. Signal transduction by the jnk group of map kinases. Cell 103 (2), 239–252.
Dharmawardhane, S., Hernandez, E., Vlaar, C., 2013. Development of ehop-016: a small molecule inhibitor of rac. Enzymes 117–146.
Dokmanovic, M., Hirsch, D.S., Shen, Y., Wu, W.J., 2009. Rac1 contributes to trastuzumab resistance of breast cancer cells: rac1 as a potential therapeutic target for the treatment of trastuzumab-resistant breast cancer. Mol. Cancer Ther. 8 (6), 1557–1569.
Espinha, G., Osaki, J.H., Magalhaes, Y.T., Forti, F.L., 2015. Rac1 gtpase-deficient hela cells present reduced DNA repair, proliferation, and survival under uv or gamma irradiation. Mol. Cell. Biochem. 404 (1), 281–297.
Etienne-Manneville, S., Hall, A., 2002. Rho gtpases in cell biology. Nature 420 (6916), 629–635.
Fang, D., Chen, H., Zhu, J.Y., Wang, W., Teng, Y., Ding, H.F., Jing, Q., Su, S.B., Huang, S., 2017. Epithelial-mesenchymal transition of ovarian cancer cells is sustained by Rac1 through simultaneous activation of MEK1/2 and Src signaling pathways. Oncogene 36 (11), 1546–1558.
Feng, H., Hu, B., Vuori, K., Sarkaria, J.N., Furnari, F.B., Cavenee, W.K., Cheng, S.Y., 2014. Egfrviii stimulates glioma growth and invasion through pka-dependent serine phosphorylation of dock180. Oncogene 33 (19), 2504–2512.
Ferri, N., Corsini, A., Bottino, P., Clerici, F., Contini, A., 2009. Virtual screening approach for the identification of new rac1 inhibitors. J. Med. Chem. 52 (14), 4087–4090.
Fritz, G., Henninger, C., 2015. Rho gtpases: novel players in the regulation of the DNA damage response? Biomolecules 5 (4), 2417–2434.
Fritz, G., Kaina, B., 2006. Rho gtpases: promising cellular targets for novel anticancer drugs. Curr. Cancer Drug Targets 6 (1), 1–14.
Fritz, G., Kaina, B., 2013. Rac1 gtpase, a multifunctional player in the regulation of genotoxic stress response. Cell Cycle 12 (16), 2521–2522.
Gao, Y., Dickerson, J.B., Guo, F., Zheng, J., Zheng, Y., 2004. Rational design and characterization of a rac gtpase-specific small molecule inhibitor. Proc. Natl. Acad. Sci. U. S. A. 101 (20), 7618–7623.
Gerlinger, M., McGranahan, N., Dewhurst, S.M., Burrell, R.A., Tomlinson, I., Swanton, C., 2014. Cancer: evolution within a lifetime. Annu. Rev. Genet. 48 (1), 215–236.
Goel Hira, L., Pursell, B., Shultz Leonard, D., Greiner Dale, L., Brekken Rolf, A., Vander Kooi Craig, W., Mercurio Arthur, M., 2016. P-rex1 promotes resistance to vegf/vegfr-targeted therapy in prostate cancer. Cell Rep. 14 (9), 2193–2208.
Gonzalez, N., Cardama, G.A., Comin, M.J., Segatori, V.I., Pifano, M., Alonso, D.F., Gomez, D.E., Menna, P.L., 2017. Pharmacological inhibition of rac1-pak1 axis restores tamoxifen sensitivity in human resistant breast cancer cells. Cell Signal. 154–161.
Gulhati, P., Bowen, K.A., Liu, J., Stevens, P.D., Rychahou, P.G., Chen, M., Lee, E.Y., Weiss, H.L., O'Connor, K.L., Gao, T., Evers, B.M., 2011. Mtorc1 and mtorc2 regulate emt, motility, and metastasis of colorectal cancer via rhoa and rac1 signaling pathways. Cancer Res. 71 (9), 3246–3256.
Halatsch, M.E., Low, S., Mursch, K., Hielscher, T., Schmidt, U., Unterberg, A., Vougioukas, V.I., Feuerhake, F., 2009. Candidate genes for sensitivity and resistance of human glioblastoma multiforme cell lines to erlotinib. Laboratory investigation. J. Neurosurg. 111 (2), 211–218.
Hazar-Rethinam, M., de Long, L.M., Gannon, O.M., Boros, S., Vargas, A.C., Dzienis, M., Mukhopadhyay, P., Saenz-Ponce, N., Dantzic, D.D.E., Simpson, F., Saunders, N.A., 2015. Racgap1 is a novel downstream effector of e2f7-dependent resistance to doxorubicin and is prognostic for overall survival in squamous cell carcinoma. Mol. Cancer Ther. 14 (8), 1939–1950.
Hein, A.L., Post, C.M., Sheinin, Y.M., Lakshmanan, I., Natarajan, A., Enke, C.A., Batra, S.K., Ouellette, M.M., Yan, Y., 2016. Rac1 gtpase promotes the survival of breast cancer cells in response to hyper-fractionated radiation treatment. Oncogene 35 (49), 6319–6329.
Hinde, E., Yokomori, K., Gaus, K., Hahn, K.M., Gratton, E., 2014. Fluctuation-based imaging of nuclear rac1 activation by protein oligomerisation. Sci. Rep. 4219.
Hodis, E., Watson, I.R., Kryukov, G.V., Arold, S.T., Imielinski, M., Theurillat, J.P., Nickerson, E., Auclair, D., Li, L., Place, C., Dicara, D., et al., 2012. A landscape of driver mutations in melanoma. Cell 150 (2), 251–263.
Hofbauer, S.W., Krenn, P.W., Ganghammer, S., Asslaber, D., Pichler, U., Oberascher, K., Henschler, R., Wallner, M., Kerschbaum, H., Greil, R., Hartmann, T.N., 2014. Tiam1/rac1 signals contribute to the proliferation and chemoresistance, but not motility, of chronic lymphocytic leukemia cells. Blood 123 (14), 2181–2188.
Holm, C., Rayala, S., Jirstrom, K., Stal, O., Kumar, R., Landberg, G., 2006. Association between pak1 expression and subcellular localization and tamoxifen resistance in breast cancer patients. J. Natl. Cancer Inst. 98 (10), 671–680.
Holm, C., Kok, M., Michalides, R., Fles, R., Koornstra, R.H., Wesseling, J., Hauptmann, M., Neefjes, J., Peterse, J.L., Stal, O., Landberg, G., et al., 2009. Phosphorylation of the oestrogen receptor alpha at serine 305 and prediction of tamoxifen resistance in breast cancer. J. Pathol. 217 (3), 372–379.
Holohan, C., Van Schaeybroeck, S., Longley, D.B., Johnston, P.G., 2013. Cancer drug resistance: an evolving paradigm. Nat. Rev. Cancer 13 (10), 714–726.
Huelsenbeck, S.C., Schorr, A., Roos, W.P., Huelsenbeck, J., Henninger, C., Kaina, B., Fritz, G., 2012. Rac1 protein signaling is required for DNA damage response stimulated by topoisomerase ii poisons. J. Biol. Chem. 287 (46), 38590–38599.
Irwin, J.J., Shoichet, B.K., 2005. Zinc–a free database of commercially available compounds for virtual screening. J. Chem. Inf. Model. 45 (1), 177–182.
Jaffe, A.B., Hall, A., 2005. Rho gtpases: biochemistry and biology. Annu. Rev. Cell Dev. Biol. 21, 247–269.
Jordan, V.C., 2008. Tamoxifen: catalyst for the change to targeted therapy. Eur. J. Cancer 44 (1), 30–38.
Jung, J.-W., Hwang, S.-Y., Hwang, J.-S., Oh, E.-S., Park, S., Han, I.-O., 2007. Ionising radiation induces changes associated with epithelial-mesenchymal transdifferentiation and increased cell motility of a549 lung epithelial cells. Eur. J. Cancer 43 (7), 1214–1224.
Kaneto, N., Yokoyama, S., Hayakawa, Y., Kato, S., Sakurai, H., Saiki, I., 2014. Rac1 inhibition as a therapeutic target for gefitinib-resistant non-small-cell lung cancer. Cancer Sci. 105 (7), 788–794.
Karpel-Massler, G., Schmidt, U., Unterberg, A., Halatsch, M.-E., 2009. Therapeutic inhibition of the epidermal growth factor receptor in high-grade gliomas: where do we

stand? Mol. Cancer Res. 7 (7), 1000–1012.
Karpel-Massler, G., Westhoff, M.-A., Zhou, S., Nonnenmacher, L., Dwucet, A., Kast, R.E., Bachem, M.G., Wirtz, C.R., Debatin, K.-M., Halatsch, M.-E., 2013. Combined inhibition of her1/egfr and rac1 results in a synergistic antiproliferative effect on established and primary cultured human glioblastoma cells. Mol. Cancer Ther. 12 (9), 1783–1795.
Karpel-Massler, G., Westhoff, M.A., Kast, R.E., Dwucet, A., Karpel-Massler, S., Nonnenmacher, L., Siegelin, M.D., Wirtz, C.R., Debatin, K.M., Halatsch, M.E., 2017. Simultaneous interference with her1/egfr and rac1 signaling drives cytostasis and suppression of survivin in human glioma cells in vitro. Neurochem. Res. 42 (5), 1543–1554.
Krauthammer, M., Kong, Y., Ha, B.H., Evans, P., Bacchiocchi, A., McCusker, J.P., Cheng, E., Davis, M.J., Goh, G., Choi, M., Ariyan, S., et al., 2012. Exome sequencing identifies recurrent somatic rac1 mutations in melanoma. Nat. Genet. 44 (9), 1006–1014.
Lemmon, M.A., Schlessinger, J., 2010. Cell signaling by receptor-tyrosine kinases. Cell 141 (7), 1117–1134.
Mardilovich, K., Olson, M.F., Baugh, M., 2012. Targeting rho gtpase signaling for cancer therapy. Future Oncol. 8 (2), 165–177.
Marei, H., Malliri, A., 2016. Rac1 in human diseases: the therapeutic potential of targeting rac1 signaling regulatory mechanisms. Small GTPases 1–25.
Morrison, G., Fu, X., Shea, M., Nanda, S., Giuliano, M., Wang, T., Klinowska, T., Osborne, C.K., Rimawi, M.F., Schiff, R., 2014. Therapeutic potential of the dual egfr/her2 inhibitor azd8931 in circumventing endocrine resistance. Breast Cancer Res. Treat. 144 (2), 263–272.
Motwani, M., Li, D.Q., Horvath, A., Kumar, R., 2013. Identification of novel gene targets and functions of p21-activated kinase 1 during DNA damage by gene expression profiling. PLoS One 8 (8), e66585.
Murray, B.W., Guo, C., Piraino, J., Westwick, J.K., Zhang, C., Lamerdin, J., Dagostino, E., Knighton, D., Loi, C.-M., Zager, M., Kraynov, E., et al., 2010. Small-molecule p21-activated kinase inhibitor pf-3758309 is a potent inhibitor of oncogenic signaling and tumor growth. Proc. Natl. Acad. Sci. 107 (20), 9446–9451.
Musgrove, E.A., Sutherland, R.L., 2009. Biological determinants of endocrine resistance in breast cancer. Nat. Rev. Cancer 9 (9), 631–643.
Nheu, T.V., He, H., Hirokawa, Y., Tamaki, K., Florin, L., Schmitz, M.L., Suzuki-Takahashi, I., Jorissen, R.N., Burgess, A.W., Nishimura, S., Wood, J., et al., 2002. The k252a derivatives, inhibitors for the pak/mlk kinase family selectively block the growth of ras transformants. Cancer J. 8 (4), 328–336.
Nicholson, R.I., Gee, J.M.W., Harper, M.E., 2001. Egfr and cancer prognosis. Eur. J. Cancer 37 (Suppl. 4), 9–15.
Nohata, N., Uchida, Y., Stratman, A.N., Adams, R.H., Zheng, Y., Weinstein, B.M., Mukouyama, Y.-S., Gutkind, J.S., 2016. Temporal-specific roles of rac1 during vascular development and retinal angiogenesis. Dev. Biol. 411 (2), 183–194.
Orgaz, J.L., Herraiz, C., Sanz-Moreno, V., 2014. Rho gtpases modulate malignant transformation of tumor cells. Small GTPases e29019.
Pandey, A., Podtelejnikov, A.V., Blagoev, B., Bustelo, X.R., Mann, M., Lodish, H.F., 2000. Analysis of receptor signaling pathways by mass spectrometry: identification of vav-2 as a substrate of the epidermal and platelet-derived growth factor receptors. Proc. Natl. Acad. Sci. U. S. A. 97 (1), 179–184.
Paplomata, E., O'Regan, R., 2014. The pi3k/akt/mtor pathway in breast cancer: targets, trials and biomarkers. Ther. Adv. Med. Oncol. 6 (4), 154–166.
Petkovic, M., Vrdoljak, E., Ruzic, I.P., Belev, B., Omrcen, T., Ledina, D., Tomek, R., Ruzic, B., Situm, M., Buca, A., Pisac, V.P., 2012. Clinical recommendations for treating and monitoring patients with renal cancer. Lijec. Vjesn. 134 (1–2), 5–8.
Porchia, L.M., Guerra, M., Wang, Y.-C., Zhang, Y., Espinosa, A.V., Shinohara, M., Kulp, S.K., Kirschner, L.S., Saji, M., Chen, C.-S., Ringel, M.D., 2007. 2-amino- < em > n < /em > -{4-[5-(2-phenanthrenyl)-3-(trifluoromethyl)-1 < em > h < /em > -pyrazol-1-yl]-phenyl} acetamide (osu-03012), a celecoxib derivative, directly targets p21-activated kinase. Mol. Pharmacol. 72 (5), 1124–1131.
Ramos, P., Bentires-Alj, M., 2015. Mechanism-based cancer therapy: resistance to therapy, therapy for resistance. Oncogene 34 (28), 3617–3626.
Ramsay, A.G., Clear, A.J., Fatah, R., Gribben, J.G., 2012. Multiple inhibitory ligands induce impaired t-cell immunologic synapse function in chronic lymphocytic leukemia that can be blocked with lenalidomide: establishing a reversible immune evasion mechanism in human cancer. Blood 120 (7), 1412–1421.
Rau, K.M., Kang, H.Y., Cha, T.L., Miller, S.A., Hung, M.C., 2005. The mechanisms and managements of hormone-therapy resistance in breast and prostate cancers. Endocr. Relat. Cancer 12 (3), 511–532.
Razavi, S.-M., Lee, K.E., Jin, B.E., Aujla, P.S., Gholamin, S., Li, G., 2016. Immune evasion strategies of glioblastoma. Front. Surg. 11.
Ridley, A.J., 2015. Rho gtpase signalling in cell migration. Curr. Opin. Cell Biol. 36, 103–112.
Risbridger, G.P., Davis, I.D., Birrell, S.N., Tilley, W.D., 2010. Breast and prostate cancer: more similar than different. Nat. Rev. Cancer 10 (3), 205–212.
Rolfo, C., Sortino, G., Smits, E., Passiglia, F., Bronte, G., Castiglia, M., Russo, A., Santos, E.S., Janssens, A., Pauwels, P., Raez, L., 2014. Immunotherapy: is a minor god yet in the pantheon of treatments for lung cancer? Expert Rev. Anticancer Ther. 14 (10), 1173–1187.
Rosenblatt, A.E., Garcia, M.I., Lyons, L., Xie, Y., Maiorino, C., Désiré, L., Slingerland, J., Burnstein, K.L., 2011. Inhibition of the rho gtpase, rac1, decreases estrogen receptor levels and is a novel therapeutic strategy in breast cancer. Endocr. Relat. Cancer 18 (2), 207–219.
Scott, D.E., Bayly, A.R., Abell, C., Skidmore, J., 2016. Small molecules, big targets: drug discovery faces the protein-protein interaction challenge. Nat. Rev. Drug Discov. 15 (8), 533–550.
Shin, J.-Y., Wey, M., Umutesi, H.G., Sun, X., Simecka, J., Heo, J., 2016. Thiopurine prodrugs mediate immunosuppressive effects by interfering with rac1 protein function. J. Biol. Chem. 291 (26), 13699–13714.
Shutes, A., Onesto, C., Picard, V., Leblond, B., Schweighoffer, F., Der, C.J., 2007. Specificity and mechanism of action of eht 1864, a novel small molecule inhibitor of rac family small gtpases. J. Biol. Chem. 282 (49), 35666–35678.
Skvortsov, S., Dudas, J., Eichberger, P., Witsch-Baumgartner, M., Loeffler-Ragg, J., Pritz, C., Schartinger, V.H., Maier, H., Hall, J., Debbage, P., Riechelmann, H., et al., 2014. Rac1 as a potential therapeutic target for chemo-radioresistant head and neck squamous cell carcinomas (hnscc). Br. J. Cancer 110 (11), 2677–2687.
Sylow, L., Nielsen, I.L., Kleinert, M., Moller, L.L., Ploug, T., Schjerling, P., Bilan, P.J., Klip, A., Jensen, T.E., Richter, E.A., 2016. Rac1 governs exercise-stimulated glucose uptake in skeletal muscle through regulation of glut4 translocation in mice. J. Physiol. 594 (17), 4997–5008.
Tao, J., Oladimeji, P., Rider, L., Diakonova, M., 2011. Pak1-nck regulates cyclin d1 promoter activity in response to prolactin. Mol. Endocrinol. 25 (9), 1565–1578.
Thiery, J.P., Acloque, H., Huang, R.Y., Nieto, M.A., 2009. Epithelial-mesenchymal transitions in development and disease. Cell 139 (5), 871–890.
Vigil, D., Cherfils, J., Rossman, K.L., Der, C.J., 2010. Ras superfamily gefs and gaps: validated and tractable targets for cancer therapy? Nat. Rev. Cancer 10 (12), 842–857.
Vu, H.L., Rosenbaum, S., Purwin, T.J., Davies, M.A., Aplin, A.E., 2015. Rac1 p29 s regulates pd-l1 expression in melanoma. Pigment Cell Melanoma Res. 28 (5), 590–598.
Wang, C.Y., Liu, P.Y., Liao, J.K., 2008. Pleiotropic effects of statin therapy: molecular mechanisms and clinical results. Trends Mol. Med. 14 (1), 37–44.
Wang, J.-Y., Yu, P., Chen, S., Xing, H., Chen, Y., Wang, M., Tang, K., Tian, Z., Rao, Q., Wang, J., 2013. Activation of rac1 gtpase promotes leukemia cell chemotherapy resistance, quiescence and niche interaction. Mol. Oncol. 7 (5), 907–916.
Wang, M., Zhang, C., Song, Y., Wang, Z., Wang, Y., Luo, F., Xu, Y., Zhao, Y., Wu, Z., Xu, Y., 2017. Mechanism of immune evasion in breast cancer. Onco Targets Ther. 1561–1573.
Watson, I.R., Li, L., Cabeceiras, P.K., Mahdavi, M., Gutschner, T., Genovese, G., Wang, G., Fang, Z., Tepper, J.M., Stemke-Hale, K., Tsai, K.Y., et al., 2014. The rac1 p29 s hotspot mutation in melanoma confers resistance to pharmacological inhibition of raf. Cancer Res. 74 (17), 4845–4852.
Westin, J.R., 2015. Busting robustness: using cancer's greatest strength to our advantage. Future Oncol. 11 (1), 73–77.
Wong, A.J., Ruppert, J.M., Bigner, S.H., Grzeschik, C.H., Humphrey, P.A., Bigner, D.S., Vogelstein, B., 1992. Structural alterations of the epidermal growth factor receptor gene in human gliomas. Proc. Natl. Acad. Sci. U. S. A. 89 (7), 2965–2969.
Wu, A., Loutherback, K., Lambert, G., Estevez-Salmeron, L., Tlsty, T.D., Austin, R.H., Sturm, J.C., 2013. Cell motility and drug gradients in the emergence of resistance to chemotherapy. Proc. Natl. Acad. Sci. U. S. A. 110 (40), 16103–16108.
Yan, Y., Greer, P.M., Cao, P.T., Kolb, R.H., Cowan, K.H., 2012. Rac1 gtpase plays an important role in gamma-irradiation induced g2/m checkpoint activation. Breast Cancer Res.: BCR 14 (2), R60.
Yan, Y., Hein, A.L., Etekpo, A., Burchett, K.M., Lin, C., Enke, C.A., Batra, S.K., Cowan, K.H., Ouellette, M.M., 2014. Inhibition of rac1 gtpase sensitizes pancreatic cancer cells to gamma-irradiation. Oncotarget 5 (21), 10251–10270.
Yoon, C.-H., Hyun, K.-H., Kim, R.-K., Lee, H., Lim, E.-J., Chung, H.-Y., An, S., Park, M.-J., Suh, Y., Kim, M.-J., Lee, S.-J., 2011. The small gtpase rac1 is involved in the maintenance of stemness and malignancies in glioma stem-like cells. FEBS Lett. 585 (14), 2331–2338.
Yoon, C., Cho, S.J., Chang, K.K., Park, D.J., Ryeom, S.W., Yoon, S.S., 2017. Role of rac1 pathway in epithelial-to-mesenchymal transition and cancer stem-like cell phenotypes in gastric adenocarcinoma. Mol. Cancer Res. 15 (8), 1106–1116.
Zhao, Y., Wang, Z., Jiang, Y., Yang, C., 2011. Inactivation of rac1 reduces trastuzumab resistance in pten deficient and insulin-like growth factor i receptor overexpressing human breast cancer skbr3 cells. Cancer Lett. 313 (1), 54–63.
Zhou, Y.-C., Liu, J.-Y., Li, J., Zhang, J., Xu, Y.-Q., Zhang, H.-W., Qiu, L.-B., Ding, G.-R., Su, X.-M., Mei, S., Guo, G.-Z., 2011. Ionizing radiation promotes migration and invasion of cancer cells through transforming growth factor-beta–mediated epithelial–mesenchymal transition. Int. J. Radiat. Oncol. Biol. Phys. 81 (5), 1530–1537.
Zhou, T., Wang, C.H., Yan, H., Zhang, R., Zhao, J.B., Qian, C.F., Xiao, H., Liu, H.Y., 2016. Inhibition of the rac1-wave2-arp2/3 signaling pathway promotes radiosensitivity via downregulation of cofilin-1 in u251 human glioma cells. Mol. Med. Rep. 13 (5), 4414–4420.
Zhu, G., Fan, Z., Ding, M., Zhang, H., Mu, L., Ding, Y., Zhang, Y., Jia, B., Chen, L., Chang, Z., Wu, W., 2015. An egfr/pi3k/akt axis promotes accumulation of the rac1-gef tiam1 that is critical in egfr-driven tumorigenesis. Oncogene 34 (49), 5971–5982.
Zins, K., Lucas, T., Reichl, P., Abraham, D., Aharinejad, S., 2013. A rac1/cdc42 gtpase-specific small molecule inhibitor suppresses growth of primary human prostate cancer xenografts and prolongs survival in mice. PLoS One 8 (9), e74924.