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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