

Review

The role of RhoC in malignant tumor invasion, metastasis and targeted therapy

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Summary. As an important member of the RhoGTPase family, RhoC has various biological functions, such as regulating cytoskeleton reorganization, influencing cell adhesion, and migration. During recent decades, RhoC has been proven to be involved in the invasion and metastasis of malignant tumor and is thus a promising target of tumor therapy. This review focuses on the molecular mechanism of RhoC in invasion and metastasis of malignant tumors, as well as its research prospects as a potential target for tumor therapy.

Key words: RhoC, Malignant tumor, Invasion, Metastasis, Targeted therapy

Introduction

RhoC (Ras homolog gene family, member C) is an important member of the RhoGTPase family, which belongs to the Ras superfamily (Wherlock and Mellor, 2002). It is generally accepted that the RhoGTPase family can be classified into 20 subfamilies according to their amino acids sequence and specific structures, including Rho (RhoA, RhoB, and RhoC), Rac, Cdc42, Rnd, RhoD, and TTF (Prendergast et al., 1995). The Rho family has important biological functions, such as regulating cytoskeletal reorganization, and participating in cell proliferation, differentiation, apoptosis, cell adhesion, and migration (Ridley, 2001; Jaffe and Hall, 2005). Among them, RhoC has been reported to play an

important role in the invasion and metastasis of melanoma (Clark et al., 2001), gastric cancer (Liu et al., 2007), esophageal squamous cell carcinoma (Faried et al., 2006), and other malignant tumors, which aroused widespread interest among researchers (Hakem et al., 2005; Mukai et al., 2006). In this review, we discuss the vital role of RhoC in the molecular mechanism of invasion and metastasis, and highlight the potential of RhoC-targeted molecular therapy in malignant tumors.

Similar to other members of the Ras superfamily, RhoC is a small molecular weight GTPase, whose gate is switched on when it binds with GTP and closed when it binds with GDP. Its activity is determined mainly by the ratio of guanine nucleotide exchange factors (GEFs) to GTPase activating proteins (GAPs). GEFs catalyze the phosphorylation of RhoGDP into active RhoGTP, and induce Rho located in the cell membrane to regulate its downstream effectors and exert its biological functions. In contrast, GAP hydrolyzes and inactivates RhoGTP (Bos et al., 2007).

RhoC participates in the invasion and metastasis of malignant tumors

The development and progression of a tumor involves multiple steps, such as abnormal enhancement of tumor cell proliferation, decrease in cell adhesion on the surface of the tumor cells, hydrolysis of the extracellular matrix, cell migration, lymphangiogenesis, and escape from immune killing. Liotta (2016) suggested that the invasion and metastasis of cancer cells can be divided into three stages at the molecular level: decreased cell adhesion on the surfaces of tumor cells, degradation of extracellular matrix proteins, and the migration of cancer cells via matrix remodeling.

RhoC and epithelial-mesenchymal transition (EMT)

Typically, epithelial cells are characterized by apical-basolateral polarity established by adhesion and tight junctions. Consequently, the invasion process of epithelial cancer is initiated mainly by the destruction of the integrity of the epithelium. Malignant cells subsequently destroy the basement membrane and invade the matrix layer. With the dissolution of tight cell junctions and the loss of apical-basolateral polarity, epithelial cancer cells become spindle-shaped and gain invasion and migration abilities resembling mesenchymal cells. This process is termed the epithelial–mesenchymal transition (EMT), which is related closely to cell invasion, metastasis, and chemotherapy drug resistance (Thiery, 2003). During the EMT process, epithelial cancer cells usually have decreased levels of the cell-adhesion protein E-cadherin, and begin to express mesenchymal markers, such as vimentin and N-cadherin (Singh and Settleman, 2010).

Bellovin et al. (2006) found that Ets-1, a member of the Ets transcription factor family, could act directly on the RhoC gene promoter during EMT in colon carcinoma, which led to a significant increase in RhoC expression. This was accompanied by the loss of E-cadherin in epithelial cells and the appearance of mesenchymal cell characteristics. Further statistical analysis showed that the elevated expression of RhoC was associated with poor prognosis in patients with colon cancer, and with the abnormal expression and localization of E-cadherin. Gou et al. (2014) also investigated the process of EMT induced by transforming growth factor beta 1 (TGF- β 1) or Vascular endothelial growth factor (VEGF) in ovarian cancer epithelial cells, and observed that it relied on RhoC's direct effect on the formation of lamellipodia, resulting in enhanced cancer cell migration and invasion.

RhoC and extracellular matrix degradation

The physical degradation of the cell barrier, such as the extracellular matrix (ECM) and basement membrane, along with changes to intercellular and cell-ECM cell junctions, is necessary for the process of tumor invasion and metastasis. Mounting evidence suggests that RhoC could control the expression and secretion of matrix metalloproteinases (MMPs), which affect extracellular matrix remodeling and participate in tumor cell invasion (Lozano et al., 2003). MMPs belong to a zinc finger-dependent endopeptidase family, and play an important role in regulating the dynamic structure of the ECM. During the process of cell invasion, MMPs, such as MMP2 and MMP9, are localized on the surface of invasive protrusions, marking the degradation sites of the ECM. Following the degradation of ECM components via MMPs' proteolytic activity, cancer cells expand locally and infiltrate into the peripheral blood vessels (Wiegand et al., 2005; Gialeli et al., 2011). Therefore, MMPs promote cancer cell invasion and are

involved in tumor metastasis.

Ikoma et al. (2004) reported that high levels of RhoC in pulmonary carcinoma could increase the expression and activity of MMP2 and MMP9 significantly, promoting tumor metastasis and invasion. *In vivo* mouse model experiments showed that overexpression of RhoC increased lesions of lung cancer metastases significantly. Similarly, Xie et al. (2013) suggested that the expression of MMP2, MMP9, and VEGF increased after overexpression of RhoC in hepatocellular carcinoma cells. Consistent with this result, our study in ovarian cancer also found that interfering with RhoC expression could inhibit the expression of MMP9 significantly, suggesting that RhoC promoted cancer cell metastasis through the degradation of ECM mediated by MMP9 (Zhao et al., 2010).

RhoC regulates cancer cell migration

It is generally accepted that cancer invasion into other tissues depends mainly on cell migration, including the formation and extension of protrusions, cytoskeleton contraction, and changes in cell-cell and cell-ECM adhesion.

First, cancer cells extend to form locomotory protrusions and invasive protrusions. The invasive protrusions are rich in actin, through which cancer cells penetrate the ECM and invade the peripheral blood vessels. It has been proven that RhoC/p190RhoGEF/p190RhoGAP molecules are involved directly in cell invasion induced by invasive protrusions and locomotory protrusions (Bravo-Cordero et al., 2014). For the locomotory protrusions, RhoC can inhibit Rac1 activity around the cell membrane, thus restricting the extension of locomotory protrusions and maintaining the polarity of cells. Silencing RhoC leads to an increase in the cell extension area and broadening of locomotory protrusions (Vega et al., 2011). Furthermore, the formation of invasive protrusions depends mainly on the regulation of the actin cytoskeleton by RhoC via the cofilin pathway. Silencing RhoC leads to abnormally structured invasive protrusions that lack multiple branches, which are unable to promote cell invasion into the ECM. This process relies on the specific mechanism involving p190RhoGEF/p190RhoGAP regulation: p190RhoGEF is localized at the periphery of the invasive protrusions, while p190RhoGAP is localized inside them. Clearly, both molecules have different effects on RhoC activity. As a result, the locally active RhoC around the invasive protrusions limits cofilin activity within the structure. Therefore, RhoC mediates the formation of polarized invasive protrusions through the cofilin pathway, which is spatially dependent and transient, and plays an important role in the process of invasion (Bravo-Cordero et al., 2011).

Rho-associated protein kinase (ROCK) has been recognized widely to regulate the cytoskeleton and affect cell contraction. RhoC has a high affinity for ROCK, thus allowing it to more easily induce cancer cell

invasion and metastasis (Benitah et al., 2004). ROCK phosphorylates LIM kinase and inactivates its cofilin inhibition and actin depolymerization functions (Bernard, 2006). Moreover, ROCK can phosphorylate and inactivate actin phosphatase, resulting in reduced MLC2 dephosphorylation and enhanced myosin II ATPase activity on the interaction of actin filaments, thereby increasing cell contractility (Riento and Ridley, 2003; Wilkinson et al., 2005).

Furthermore, Vega et al. (2011) found that RhoC could act on the formin family member FMNL3, which accelerates actin polymerization and assembles the microfilament skeleton by nucleation (Chesarone et al., 2009), thus restricting the expansion of locomotory protrusions and maintaining their morphological polarity (Vega et al., 2011). Kitzing et al. (2010) performed 3-dimensional cell migration experiments in the breast cancer cell line MDA-MB-435 and showed that RhoC could also bind specifically to the formin family member FMNL2 to induce cell amoeboid migration.

RhoC and tumor vasculogenesis and angiogenesis

As a tumor develops, it is necessary to absorb nutrients continuously from the outside. Vasculogenesis and angiogenesis are essential for tumor progression, which not only benefit the growth of cancer, but also create conditions for invasion and metastasis.

Rearrangement of the cytoskeleton in endothelial cells (EC) is essential for angiogenesis. Under the effects of angiogenic stimulating factors, ECs change their morphology and movement via cytoskeletal reorganization, allowing cancer cells to migrate more easily into the periventricular space (Carmeliet and Jain, 2000). It has been reported that RhoC plays an important role in the trans-endothelial migration (TEM) of cancer cells. RhoC regulates tumor cell processes that extend along the body of ECs, allowing their early attachment to the blood vessel endothelium (Merajver and Usmani, 2005). This process is also a key step in metastasis and the subsequent growth of the tumor. By contrast, Wang et al. (2008) found that knockout of RhoC inhibited F-actin filament reorganization in ECs and affected EC sprouting, thus suppressing the remodeling of the EC skeleton and reducing angiogenesis in hepatocellular carcinoma.

In addition to the above-mentioned ECM degradation to eliminate the physical barrier, the migration of cancer cells into the perivascular space also requires the generation of a variety of angiogenic factors to stimulate angiogenesis. VEGF is a multifunctional cytokine that acts as a mitogen of ECs, promoting EC proliferation and increasing vascular permeability in the process of tumor vasculogenesis and angiogenesis (Hoepfner et al., 2015). In esophageal squamous cell carcinoma, the expression level of RhoC correlated positively with VEGF expression, and with the depth of tumor invasion and lymphatic metastasis (Zhao et al., 2015). Van Golen et al. (2000a) reported that

overexpression of RhoC not only promoted the movement of cancer cells by facilitating the formation of actin stress fibers and plaque spots, but also induced tumor angiogenesis and metastasis by upregulating VEGF and basic fibroblast growth factor (bFGF) levels in inflammatory breast cancer (IBC) (Van Golen et al., 2000b).

Therefore, RhoC promotes tumor cell angiogenesis, not only through the migration and reorganization of ECs, but also by inducing the release of VEGF.

Tumor drug therapy targeting RhoC

The spherical spatial structure and the limited effective space for the Rho protein to bind with other molecules mean that it is not suitable for the direct action of traditional drugs. Therefore, treatments targeting Rho proteins mainly focus on interfering with upstream regulatory factors or downstream effector molecules in its signal transduction pathway (Kristelly et al., 2004).

Direct regulation of RhoC GTPase activity

As mentioned previously, the activation of RhoC is determined by the ratio of RhoGEF to GAP. The former phosphorylates and activates RhoC, while the latter hydrolyzes and inactivates it; therefore, treatment targeting these two molecules can control RhoC activity directly. For example, Thomas et al. (2011) reported that Src kinase phosphorylates p190RhoGAP to reduce RhoC activity and inhibit metastasis in bladder cancer. Similarly, protein kinase C (PKC) regulates the activity of RhoGEF, Tiam1, and p115RhoGEF by direct phosphorylation. As a result, it activates RhoC and plays an important role in the related cell differentiation, apoptosis, and migration. In addition, overexpression of PKC ϵ has been observed in gastric cancer, thyroid cancer, lung cancer, and others, in which it stimulates the malignant transformation of cell morphology (Toton et al., 2011).

Moreover, Pan et al. (2006) showed that after interfering with PKC ϵ , the threonine phosphorylation level and activity of RhoC decreased significantly in squamous cell carcinoma (HNSCC) cells, and the cell migration and invasion ability were also inhibited. Further ScanProsite analysis revealed that there might be two PKC phosphorylation sites in the RhoC amino acid sequence, suggesting that RhoC is a substrate of PKC ϵ (Pan et al., 2005). Thus, PKC ϵ can also phosphorylate RhoC directly to maintain its activity and stability, allowing it to mediate cell invasion and movement.

Therefore, treatments targeting RhoC's upstream regulatory molecules, such as RhoGEF and PKC, have potential therapeutic applications.

HMG-CoA reductase inhibitor

It is generally recognized that GEF and GAP

regulate RhoC activity through the post-translational modification of the Rho protein. That is, the CAAX motifs in the catalytic subunit bind covalently to isoprenoid groups, namely farnesyl or geranylgeranyl groups (Sebti and Der, 2003).

Farnesyl pyrophosphate (FPP) and other products with prenyl groups are synthesized from Hydroxymethylglutaryl-CoA (HMG-CoA) via the mevalonate pathway and are involved in Rho isoprenyl modification. Thus, HMG-CoA reductase inhibitors suppress the post-translational activation of RhoC via this pathway (Bathaie et al., 2017). For example, Collisson et al. (2003) reported that the HMG-CoA reductase inhibitor atorvastatin could inhibit the process of geranylgeranylation, which affects the subcellular localization and activity of RhoC, thereby inhibiting metastasis of melanoma cell and reducing the risk of metastasis and recurrence in patients. Islam et al. (2013) found that atorvastatin treatment reduced the levels of p-ERK1/2 and p-STAT3 by suppressing the activity of RhoC, and inhibited cell viability, proliferation, and invasion significantly, as well as angiogenesis and lung metastasis in head and neck cancer.

Another HMG-CoA reductase inhibitor, rosuvastatin, is also able to reduce cortical actin organization through the Rho protein, increasing tubulin rupture and reducing integrin-mediated focal adhesion, thus inhibiting the metastatic ability of breast cancer F311 cells (Farina et al., 2002).

Therefore, the mechanism by which statins inhibit tumor invasion and metastasis depends mainly on the activity of Rho proteins, especially RhoC, resulting in changes to the cytoskeleton reorganization and regulating cell adhesion and migration. Based on this mechanism, statins represent promising cancer therapeutic adjuvants.

Prenyl function inhibitors

In addition to the above-mentioned HMG-CoA reductase inhibitors, it is also possible to use farnesol or geranylgeraniol analogs, also known as Prenyl Function Inhibitors (PFIs), as substrates for farnesyltransferase (FTase) or geranylgeranyltransferases (GGTase) to compete with the Rho protein for binding to FTase or GGTase, thus inhibiting RhoC activation. Chen et al. (2014) proposed two isoprenyl functional inhibitors in cancer therapy, termed anilinogeraniol (AGOH) and anilino-farnesol (AFOH). Both of them could inhibit the activation of RhoC under stimulation by the lipid mediator, lysophosphatidic acid (LPA), and epidermal growth factor (EGF), further blocking the invasive ability of breast cancer.

MicroRNA and RhoC—regulation at the post-transcriptional level

MicroRNAs (miRNAs) are small endogenous non-coding RNAs. They bind specifically to the 3'-

untranslated region (UTR) of their target mRNA to form an RNA silencing-inducing complex (RISC), which blocks mRNA translation or induces its degradation. Thus, miRNAs are involved in gene expression regulation at the post-transcriptional level (Tutar et al., 2015). There is increasing research interest in the relationship between miRNAs and the development and progression of tumors. MiRNAs exhibit oncogenic or tumor suppressor effects by regulating the expression of related genes, and are involved in the malignant transformation of cells, as well as the proliferation, apoptosis, invasion, and metastasis of tumor cells (Ambros, 2004).

The regulation of Rho family expression by microRNAs plays an important role in tumor development and progression. MiR-493 reduced the motility and migration ability of cells by downregulating RhoC expression in bladder cancer (Ueno et al., 2012). Our team found that miR-93-5P (Chen et al., 2015a), miR-106b (Chen et al., 2015b), and miR-519d (Sang et al., 2017) inhibited the migration and invasion of cancer cells by downregulating the expression of RhoC. Dual luciferase reporters showed that these three microRNAs could bind to the 3'-UTR of the RhoC gene, suggesting that they could have an effect on RhoC targeted therapy. Similarly, our study in endometrial carcinoma showed that miR-372 inhibited the proliferation, invasion, and metastasis of cancer cells by targeting RhoC (Liu et al., 2016).

In head and neck squamous cell carcinoma, suberoylanilide hydroxamic acid (SAHA) can reactivate miR-107 and miR-138, two kinds of tumor suppressor microRNAs targeting RhoC. A novel treatment comprising SAHA combined with the traditional chemotherapy drug cisplatin has been assessed in a clinical trial and achieved some success (Datta et al., 2016). Therefore, exploring treatment via miRNAs targeting RhoC might provide a new avenue for the molecular targeted therapy of tumors.

Conclusion

In this review, we highlighted RhoC's important role in the molecular mechanisms of invasion and metastasis of malignant tumors by influencing EMT, involving extracellular matrix degradation, and regulating cell migration and tumor angiogenesis. We further summarized the current research progress of RhoC as target for tumor therapy. Although these new tumor molecular targeted treatments are still in their infancy, these targets from basic research might also have a problem with druggability (Lin and Zheng, 2015), and their precise functions and mechanisms have not yet been investigated thoroughly. In particular, some targets are involved in various signal pathways forming complex "crosstalk" networks, such that simply interfering with one aspect might lead to other changes in normal biological function. Fortunately, with the development of biosynthetic drug technology and

increasing research on tumor molecular biology, there is reason to believe that a variety of molecular therapies targeting RhoC, combined with conventional anti-cancer treatments, might have therapeutic potential and provide new ideas for individual molecular therapy. Therefore, RhoC targeted therapy to suppress the invasion and metastasis of malignant tumors remains to be further explored and studied for clinical applications.

Acknowledgements. This work was supported by the Natural Scientific Foundation of China (Nos. 81472440; 81602266).

Conflicts of interest. The authors have no conflicts of interest to declare.

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