Roles of Rap1 signaling in tumor cell migration and invasion

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ABSTRACT

Ras-associated protein-1 (Rap1), a small GTPase in the Ras-related protein family, is an important regulator of basic cellular functions (e.g., formation and control of cell adhesions and junctions), cellular migration, and polarization. Through its interaction with other proteins, Rap1 plays many roles during cell invasion and metastasis in different cancers. The basic function of Rap1 is straightforward; it acts as a switch during cellular signaling transduction and regulated by its binding to either guanosine triphosphate (GTP) or guanosine diphosphate (GDP). However, its remarkably diverse function is rendered by its interplay with a large number of distinct Rap guanine nucleotide exchange factors and Rap GTPase activating proteins. This review summarizes the mechanisms by which Rap1 signaling can regulate cell invasion and metastasis, focusing on its roles in integrin and cadherin regulation, Rho GTPase control, and matrix metalloproteinase expression.

KEYWORDS

Tumor; metastasis; Rap1; RapGEFs; RapGAPs

Introduction

Cell migration and tumor metastasis are responsible for up to 90% of cancer-associated mortality1. Ras-associated protein-1 (Rap1) plays important roles in the regulation of multiple key events in tumor cell migration, invasion, and metastasis. Rap1, a member of the 21-kilodalton Ras-like small GTPase family, can bind to either guanosine triphosphate (GTP) or guanosine diphosphate (GDP) and is modulated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs)2,3. Rap1 shares a high degree of sequence identity (53%) with Ras protein4 and can revert the phenotype of K-Ras-transformed cells5. Consistent with this observation, overexpressed Rap1V12, a constitutively active form of Rap1 (Rap1GTP), inhibits lysophosphatidic acid (LPA)-induced Ras-dependent ERK activation6. However, Rap1 can also activate B-Raf and ERKs in a manner independent and distinct of Ras7. The many roles of Rap1 include its participation in regulation of integrin- and cadherin-mediated cell adhesion in response to various membrane receptors8 and regulation of both the recycling, avidity, and affinity of integrins by modulating an inside-out activation process9-11. Rap1 activation may promote the formation of cadherin-mediated cell-cell contacts through inside-out regulation12 or cell-cell contact-induced E-cadherin-mediated outside-in signaling13.

Regulation of Rap1 activity is primarily controlled by RapGEFs and GAPs (Figure 1). The dissociation rate of nucleotides from Rap1 is slow; however, GEFs accelerate this exchange reaction by several orders of magnitude14. Given that GEFs weaken the association between Rap1 and nucleotides, increases in GTP-bound forms over GDP-bound forms are caused by the higher intracellular concentration of GTP than GDP by approximately ten times15. GEFs contain a catalytic CDC25 homology domain and show selective activity for Rap1, although some GEFs can interact with other small G proteins16. This modulation of nucleotide binding of GEFs allows GEFs to respond to diverse stimuli, resulting in spatiotemporal regulation of Rap1. For example, RapGEFs, such as Epac1 and Epac2, are directly regulated by the secondary messenger cAMP, which controls local Epac-Rap1 signaling through its cellular distribution. Epac1 activation triggers the relocation of Epac1 to the plasma membrane, activating membrane-localized Rap1 and enhancing integrin-mediated cell adhesion17. Another RapGEF, C3G, is regulated through post-translational modifications by Src and interacts with adaptor proteins of the Crk family upon activation of several receptors, including...
platelet-derived growth factor receptor and insulin receptor. Rap1-bound GTP is efficiently hydrolyzed into GDP in the presence of RapGAPs, which accelerate the GTP hydrolysis reaction by several orders of magnitude.

Two families of Rap1-specific GAPs exist: the Rap1GAP and SIPA1 families. The mechanism through which all GAPs catalyze GTP hydrolysis primarily depends on the stabilization of the catalytic machinery of G protein through insertion of a catalytic side chain into the nucleotide-binding pocket, an arginine side chain for RasGAPs and asparagine side chain for RapGAPs. Through differentially distributed subcellular features, such as protein-protein interactions and epigenetic modifications, RapGAPs target different Rap1-dependent signaling complexes and consequently perform distinct cellular functions. For example, Rap1GAP is recruited from the cytosol to the plasma membrane by its interaction with Gαz, which is activated by G protein coupled receptors. E6 oncoprotein binds to SIPA1L1 (E6TP1) and targets it for degradation, resulting in deregulation of Rap1 activity. In melanoma cells, Rap1GAP is downregulated via promoter methylation, promoting Rap1 activation, ERK phosphorylation, and cell proliferation and survival.

Moreover, the diversity of cellular functions regulated by small G proteins is determined by the distinct downstream effectors of these proteins. The effectors of Rap1 include the adaptor proteins AF-6, RAPL, Ezrin, Rasip1, Radil, Krit1, RacGEFs (e.g., Tiam1 and Vav2), and RhoGAPs, including RA-RhoGAP and Arap3, which contribute to the regulation of Rap1-dependent cellular functions, such as cell adhesion, junction, migration, and polarization. RAPL deficiency has been speculated to significantly reduce the ability of chemokine-stimulated lymphocytes to adhere to ICAM and migrate into peripheral lymph nodes and spleen. AF-6 interacts with p120 catenin and inhibits E-cadherin endocytosis in a Rap1-dependent manner, affecting E-cadherin-mediated cell-cell adhesion. Rasip1 mediates Rap1-induced cell spreading without affecting adhesion; it induces junctional tightening via interaction with Radil. Concomitantly, Rap1 promotes translocation of Radil from cytoplasm to plasma membrane, and Radil overexpression increases cell adhesion. Rap1 interacts with Tiam1 and Vav2 without affecting their catalytic activity but in turn activates Rac and CDC42, regulating cell polarization and movement. Furthermore, the Rap1 effector B-Raf can mediate ERK activation, and regulation of PI3K/Akt by Rap1 is an important mechanism in the control of cell survival and proliferation (Figure 1).

Figure 1: Mechanisms by which Rap1 signaling controls tumor cell invasion and metastasis. Rap1 signaling regulates integrin- or cadherin-mediated cell adhesion, expression levels of proteases (e.g., matrix metalloproteinase), and cytoskeletal changes, which are linked to tumor cell proliferation, invasion, and metastasis.

Tumor cell migration, invasion, and metastasis: roles of Rap1 signaling and its regulators

The diverse roles of Rap1 in the regulation of normal cell growth are translated into several distinct activities in tumor
cell development. Rap1 demonstrates distinct actions during metastasis depending on the assay employed and cancer type studied (Table 1) based on standard assays used to determine the roles of Rap1 include overexpression of wild-type Rap1 or its active mutants (Rap1V12 or Rap1E63), the use of extracellular stimuli, such as HGF, TGFβ, EGF, or cAMP analogs, and the use of siRNAs and the pharmacological inhibitor GGTI-298, followed by assessment of the invasive capacity of tumor cells by means of scratch and Transwell assays in vitro or xenograft models in vivo. Active Rap1 inhibits tumor invasion and metastasis in bladder, lung, and brain33,34, whereas it has the opposite effect in melanoma, leukemia, breast cancer, esophageal squamous cell carcinoma, head and neck squamous cell carcinoma (HNSCC), pancreatic carcinoma, and non-small cell lung carcinoma35-40. Rap1 activation promotes the adhesion of lymphoma cells to endothelial cells and its subsequent transmigration into the hematopoietic system, through which lymphoma cells spread to distant organs39. Moreover, Rap1E63 contributes to the invasive ability of prostate cancer cells41, whereas Rap1V12 suppresses prostate cancer metastasis42. Additionally, both Rap1V12 and Rap1GAP impair the migratory and invasive abilities of melanoma cells39, whereas the two isoforms of Rap1, Rap1A, and Rap1B exert the opposite effect on cell motility in glioma43,44. These manifold phenotypes reflect the multiple signaling pathways that exist downstream of Rap1.

Similar to Rap1, which plays diverse roles in tumor metastasis, Rap1 regulators are pleiotropic (Table 2). Overexpression of the Rap1 activator DOCK4 suppresses invasion of mouse osteosarcoma cells43. Targeted shRNA-mediated EPAC1 inhibition reduces pancreatic cancer cell migration and invasion46. Stable expression of a non-degradable mutant of RAPGEF2 in breast cancer cells blocks tumor invasion and metastasis47. Rap1GAP inhibits tumor cell invasion in pancreatic carcinoma, thyroid carcinoma, melanoma, renal carcinoma, and colon cancer46-50; however, increased expression of Rap1GAP induces cell invasion in leukemia51. High expression of SIPA1 promotes tumor invasion and metastasis in prostate cancer, melanoma, and breast cancer52,53. In colon cancer, downregulation of endogenous SIPA1 increases the invasive ability of cells54. This finding is inconsistent with the result for ovarian cancer, wherein C3G/Rap1 signaling promotes cell invasion, whereas Rap1GAP does not affect cell mobility55,56. Most of the studies included in Table 2 also assessed the role of Rap1 and the effect of GEFs and GAPs on tumor invasion and metastasis. Exceptions are the study on Rap1GAP in pancreatic carcinoma49 and SIPA1 in melanoma and colorectal carcinoma52,54; these studies did not assess whether Rap1 is involved in the observed cellular changes.

Other potential functions of Rap1 GEFs and GAPs in addition to their regulatory role on Rap1 activity cannot be ruled out. A recent study demonstrated that nuclear SIPA1 could activate integrin β1 promoter and promote breast cancer cell invasion in a Rap1-independent manner53. Moreover, the opposite influences of Rap1GAP and SIPA1 on regulation of melanoma cell invasion imply that there exist multiple mechanisms through which Rap1GAPs can affect cell migration and invasion. Several independent investigations have shown that the Rap1 GEF PDZ-GEF2 promotes tumor cell invasion in colon cancer, whereas Rap1GAP and SIPA1 suppresses cancer cell invasion54,57. This finding suggests a potential central role of Rap1 signaling and Rap1 signaling partners in colorectal carcinoma metastasis, and that the function of the Rap1 signaling proteins in tumor metastasis is very complex and mediates the effect of a host of other cellular and tissue-specific factors.

Dissemination of tumor cells from the original tumor mass involves a breakdown of cell-cell adhesion. Tumor cell migration is promoted by disruption of the extracellular matrix to form a proteolytic microtrack. Rap1 signaling participates in several processes that contribute to these events (Figure 2), as outlined below.

### Rap1 signaling regulates cell adhesion

Rap1 signaling regulates integrins and cadherins, which play important roles in cell adhesion to ECM and in cell-cell adhesion58. In lung cancer, cAMP-induced Epc-Rap activation suppresses TGFB- and HGF-stimulated cell migration by enhancing cell-cell adhesion54. JAM-A drives breast cancer cell migration and adhesion through activation of Rap1 and integrin β1 and formation of a complex between JAM-A, AF-6, and PDZ-GEF256. Disrupting the balance in Rap1 activity in melanoma cells via expression of Rap1V12 or Rap1GAP impairs cell adhesion and migration via the FAK-and integrin-dependent pathways59. Given that both Rap1-specific GAPs Rap1GAP and SIPA1 inhibit cell adhesion to ECM, concluding that Rap1 plays a role in the regulation of cell adhesion is reasonable59,52. In prostate cancer cells, SIPA1 promotes tumor cell invasion and metastasis at least partially by inhibiting Rap1-mediated cell adhesion to ECM42. Reduced cell-cell adhesion is required for individual cell dissemination and invasion at the leading edge of the tumor mass during epithelial mesenchymal transition (EMT), and mesenchymal-migrating tumor cells require strong cell-to-ECM adhesion, whereas amoeboid movement does not58. In terms of the specific role of Rap1 in regulating integrin activation and integrin-mediated cell adhesion, Rap1 forms a complex containing talin combined with RIAM, which
| Tumor types     | Function                                           | Signaling molecules                                      | Cell lines   | Methods used          | Reference |
|-----------------|----------------------------------------------------|----------------------------------------------------------|--------------|-----------------------|-----------|
| Melanoma        | 8CPT-2OMe-cAMP induces Rap1 activation and thus promotes cell migration. | Integrin αvβ3, ERK                                        | A375, MeWo   | Transwell             | 35        |
| Melanoma        | Rap1 and RIAM are required for melanoma cell invasion. | RIAMc, Vav2-RhoA-ROCK-MLC, ERK, Akt                      | BLM          | Transwell             | 59        |
| Lung cancer, RCC| 8CPT-2OMe-cAMP treatment and Rap1 activation inhibit TGFβ- or HGF-induced cell scattering. | /                                                        | A549, RCC10  | Live cell microscopy  | 34        |
| Breast cancer   | Rap1 activation is required for cell migration.    | Integrin β1                                              | MCF7         | Transwell             | 36        |
| HNSCC           | Rap1 enhances β-catenin-dependent cell invasion    | /                                                        | UM-SCC-1     | Transwell             | 75        |
| Pancreatic cancer| Rap1 activation is required for EGFR-mediated invasion and metastasis in vitro and in vivo. | p130CAS/Nck1                                             | FG, BXPC3    | Chick embryo metastasis model | 38        |
| Leukemia        | Rap1 activation is required for adherence to endothelial cells and transc Atheindelial migration. | /                                                        | A20          | Transwell             | 39        |
| Prostate adenocarcinoma | Rap1E63 increases cell migration and invasion. | Integrins α4, β3, αvβ3                                    | PC3          | Transwell             | 41        |
| Lung cancer     | Rap1E63 promotes cell migration.                   | /                                                        | H1299        | Scratch/Transwell     | 69        |
| Bladder carcinoma| Rap1V12 inhibits cell migration.                   | Paxillin-Crk-DOCK180 signaling complex, Rac1              | NBT-II       | Random cell migration | 33        |
| Melanoma        | Rap1V12 or Rap1GAPII inhibits tumor cell invasion and metastasis. | FAK, integrin β1                                        | B16F1, A375  | Transwell, transc Atheindelial migration, cell tracking | 43        |
| Prostate adenocarcinoma | Rap1V12 suppresses metastatic activity of tumor cell. | /                                                        | PC3          | Transwell             | 42        |
| ESCC            | Rap1A promotes cell migration and invasion.        | MMP2                                                     | EC109        | Transwell             | 76        |
| Breast cancer   | Rap1A is required for cell migration and invasion induced by lysophosphatidic acid. | β-Arrestin2, IQGAP1                                      | MDA-MB-231   | Scratch/Transwell     | 77        |
| Glioma          | Rap1A is required for PDGF-BB-stimulated chemotactic motility and 3D spheroid invasion. | /                                                        | U87          | Transwell, three-dimensional spheroid invasion assay | 45        |
| Glioma          | Rap1B suppresses migration of glioma cells.        | Rac1                                                     | GNS-3314     | Transwell             | 44        |
| Leukemia        | Rap1B is required for transc Atheindelial migration of cells. | Integrin β2                                             | CCRF-CEM     | Transendothelial migration | 40        |

RCC, renal cell carcinoma; HNSCC, head and neck squamous cell carcinoma; ESCC, esophageal squamous cell carcinoma.
| Item         | Tumor types   | Function                                                                 | Signaling molecules | Cell lines       | Methods used                  | References |
|-------------|--------------|--------------------------------------------------------------------------|---------------------|-----------------|------------------------------|------------|
| DOCK4       | Osteosarcoma | DOCK4 inhibits tumor cell invasion and metastasis.                      | Rap1                | 3081            | /                            | 46         |
| PDZ-GEF1    | Breast cancer| PDZ-GEF1 blocks cell invasion.                                           | /                   | MDA-MB-231      | Transwell                    | 49         |
| PDZ-GEF2    | Colon cancer | PDZ-GEF2 enhances cell migration.                                        | JAM-A/(AF6/PDZ-GEF2), Rap1A, Integbinβ1 | SKCO-15         | Scratch assay /               | 58         |
| C3G         | Ovarian cancer| C3G/Rap1 pathway promotes cell invasion and metastasis.                 | MMP2, MMP9         | SKOV3, HEY      | Transwell /                  | 65         |
| EPAC1       | Pancreatic cancer| EPAC1 inhibition inhibits cell migration and invasion.             | Rap1, Akt          | AsPC-1, PANC-1  | Transwell /                  | 47         |
| Rap1GAP     | Colon cancer | Rap1GAP impairs the ability of cells to spread and migrate on collagen IV. | Rap2               | HCT116          | Scratch assay /               | 78         |
| Rap1GAP     | Colon cancer | Downregulation of Rap1GAP endows tumor cells with more aggressive properties. | Rap1/2, Rac1       | HT-29, LoVo     | Scratch assay, transwell, phagocytic tracking assays / | 50         |
| Rap1GAP     | Pancreatic cancer| Rap1GAP inhibits cell invasion and metastasis.                      | FAK                 | MiaPaCa-2       | Transwell, colloidal gold random motility assays / | 61         |
| Rap1GAP     | Thyroid cancer| Rap1GAP inhibits cell migration and invasion.                         | Rap1, Rac          | WRT, FTC133, WRO | Transwell; scratch assay /    | 48         |
| Rap1GAP     | Thyroid cancer| Rap1GAP inhibits cell migration and invasion.                         | Rap1               | TPC1, Hth83     | Transwell; scratch assay /    | 79         |
| Rap1GAP     | Thyroid cancer| Rap1GAP inhibits cell motility.                                       | Rap1/2, FAK, Paxillin, Src | BCPAP, KTC-1, TPC-1 | Scratch assay /                | 51         |
| Rap1GAP     | Oropharyngeal SCC | Rap1GAP induces oropharyngeal SCC cell invasion and metastasis.       | Rap1, MMP2, MMP9   | UM-SCC-11A      | Transwell                     | 80         |
| Rap1GAP     | Melanoma      | Rap1GAP inhibits melanoma cell migration.                               | Src, Rap1, ERK     | LH              | Transwell /                  | 25         |
| Rap1GAP     | Ovarian cancer| Rap1GAP does not affect migration properties of OVCA cell lines.       | /                   | Established cell lines derived from patients | Transwell /                  | 65         |
| Rap1GAP     | RCC           | Rap1GAP inhibits cell invasion.                                         | Rap1               | RCC7            | Transwell /                  | 57         |
| Rap1GAP     | RCC           | Rap1GAP inhibits cell invasion.                                         | Integrons, cadherins | Caki-1, SN12C  | Transwell /                  | 81         |
| Rap1GAP     | Leukemia      | Rap1GAP promotes leukemia cell invasion.                                | MMP9               | HL-60           | Transwell /                  | 52         |
| SIPA1       | Prostate cancer| SIPA1 promotes prostate cancer cell invasion and metastasis.         | Brd4/ECM-related genes, Rap1 | LNCaP, PC3 | Transwell                     | 42         |
| SIPA1       | Melanoma      | SIPA1 is required for melanoma cell migration.                          | /                   | VM-1            | Transwell /                  | 53         |
| SIPA1       | Colon cancer  | SIPA1 inhibits cell migration and invasion.                             | /                   | HT115, Caco2    | Scratch assay /               | 82         |
| SIPA1       | Breast cancer | SIPA1 promotes cell migration and invasion.                             | Integrin β1/FAK/Akt/MMP9 | MDA-MB-231 | Scratch assay, Transwell / Zebrfish model | 54         |

SCC, squamous cell carcinoma; RCC, renal cell carcinoma.
However, a complete description of the roles of Rap1 in mediating cell adhesion in tumor cell invasion and metastasis requires further clarification.

Rap1 signaling modulates expression of matrix metalloproteinases (MMPs)

During tumor invasion and metastasis, MMPs degrade ECM barriers, cleave and activate target proteins, and regulate cell adhesion. In HNSCCs, Rap1 promotes nuclear localization of β-catenin, which induces TCF-dependent MMP7 transcription, thereby contributing to tumor cell invasion. Knockdown of C3G in ovarian cancer cells reduces MMP2 and MMP9 production and Rap1-GTP level. However, in HNSCCs, overexpression of Rap1GAP increases the expression levels of MMP2 and MMP9 and the invasive capacity of cells, although the role of Rap1 in this process is unclear. Overexpression of SIPA1 in prostate cancer cells reduces MMP12 expression. By contrast, SIPA1 knockdown in breast cancer cells reduces MMP9 expression through the FAK/Akt pathway.

Rap1 signaling controls Rho GTPase-mediated regulation of cytoskeletal dynamics

Several Rho family members function in actin cytoskeleton rearrangement and consequently in modulation of cell motility. Rap1 signaling can participate in motility regulation involving Rho family proteins, particularly Cdc42, Rac1, and RhoA. Rap1 associates with RacGEFs, such as Vav2 and Tiam1, to induce translocation of Vav2 and activates Rac1 to promote cell spreading. Cdc42 activation by Rap1 increases the activity of cell polarization-related protein complex, which in turn activates Rac1 through Tiam1 and subsequently enhances cell polarization. Moreover, Rap1 can interact with and activates Arap3, a RhoA GAP. During tumor metastasis, Rap1 increases the ability of melanoma cell to migrate via Vav2-dependent activation of the RhoA/ROCK/MLC pathway. In vitro overexpressed Rap1GAP inhibits Rap1, Rac1 activation, and thyroid tumor cell migration. Additionally, Rap1’s inhibitory effects on bladder cancer and glioma cell migration are intensified by reduced Rac1 activity. Rap1 signaling can regulate Rho-family protein activities either positively or negatively, causing a wide range of effects on tumor cell invasion and metastasis.

Rap1 signaling controls cell proliferation

Tumor cell growth can increase tumor volume and mass, contributing to invasion via physical pushing. An inhibitory effect of Rap1 signaling-related molecules on cell proliferation and invasion has been repeatedly observed; for instance, DOCK4 inhibits osteosarcoma and Rap1GAP inhibits pancreatic cancer, thyroid carcinoma, and melanoma cells. Additionally, SIPA1 drives both cell proliferation and invasion in melanoma cells. SIPA1-induced expression exerts little effect on primary tumor mass in prostate cancer but significantly increases both tumor cell invasion and metastasis, suggesting that SIPA1 promotes metastasis through mechanisms other than proliferation. SIPA1 knockdown impairs the invasive capacity of breast cancer cells while it enhances their proliferation. Similarly, overexpression of Rap1V12 in melanoma cells increases tumor mass but inhibits tumor metastasis in vivo. Moreover, Rap1GAP overexpression inhibits cell growth but induces MMP2- and MMP9-mediated oropharyngeal squamous carcinomas cell invasion.

Regulation of Rap1 is dependent on tissue and subcellular-specific factors

Rap1 signaling can affect metastasis in different manners depending on tumor types (Table 3). Tissue-specific protein expression in different tumor types likely contributes to the
regulation of Rap1 signaling, similar to the spatiotemporally regulated patterns of gene expression during tumor development. Indeed, Rap1 has been implicated in the activation and inhibition of ERK pathway in different cell types. cAMP-induced activation of Rap1 inhibits C-Raf-induced ERK activation. However, in neuronal cells expressing B-Raf, activated Rap1 can directly bind to B-Raf and induces downstream ERK activation. Additionally, over-activation or inactivation of Rap1 inhibits melanoma cell motility, suggesting that change in Rap1 activity is critical for the metastatic dissemination of melanoma cells. The interaction of Rap1 signaling with tissue-specific factors may explain this considerably diverse functions of Rap1. For example, while basal level of Rap1-GTP maintains cell adhesion, insulin-like growth factor type I receptor transiently regulates Rap1 activity through C3G and Rap1GAP to promote cell movement.

Protein subcellular localization of Rap1 is vital to the specificity and diversity of its function. Relatedly, tumor cell dissemination and invasion depends on the stability and activity of Rap1. Rap1 phosphorylation prevents the membrane association of Rap1, resulting in cytosolic and nuclear accumulation and in subsequent decrease in Rap1-dependent cell adhesion. In addition, Rap1 stabilizes β-catenin in the nucleus and enhances β-catenin-dependent transcription and invasion in HNSCC. SIPA1, recruited by AF6 and co-localized with Rap1 at cell adhesion sites, inhibits endogenous Rap1GTP and integrin β1-mediated cell adhesion to fibronectin. However, nuclear-localized SIPA1 activates the integrin β1 gene promoter and promotes cell invasion and adhesion. Novel targets for the prevention of metastasis: insights from related studies on Rap1 signaling

Prevention or early detection of the initial dissemination of tumor cells and secondary spread of tumor is an important goal in research aiming to find better clinical therapies. In a melanoma metastasis model, six distinct Rap1-regulating molecules were used to predict the aggressive capability of melanoma cells. Several inhibitors of cell motility, such as metallocaptoproteinase inhibitor and the fascin inhibitor Migrastatin, have been suggested to demonstrate clinical utility in preventing tumor cell dissemination and subsequent invasion and metastasis. However, formation of metastases often occurs prior to the diagnosis of cancer. The Rap1 signaling pathway offers many targets for novel clinical tools given that Rap1 affects not only cell polarity and cell adhesion but also cell proliferation and invasion. Treatment

| Table 3 Bidirectional effects of Rap1 signaling in different tumor types |
|-----------------------------|-----------------------------|
| Item | Metastasis-promoting tumor | Metastasis-suppressing tumor |
| Rap1 | Breast, esophageal, glioma, HNSCC, leukemia, lung, melanoma, pancreas, prostate, ovarian | Bladder, glioma, lung, prostate |
| GEFs | Colon, pancreas, ovarian | Breast, osteosarcoma |
| GAPs | Breast, leukemia, melanoma, prostate, oropharyngeal SCC | Colon, kidney, melanoma, pancreas, thyroid |

HNSCC: head and neck squamous cell carcinoma; SCC: squamous cell carcinoma.

Figure 3 Subcellular localization of Rap1 and SIPA1 during tumor cell invasion and metastasis. Subcellular localization of Rap1 (A) and SIPA1 (B) contributes to their distinct functions within a cell.
with the demethylating agent 5-aza-2′-deoxycytidine induces Rap1GAP expression and reduces melanoma cell proliferation and survival. In addition, treatment with 5-aza-deoxycytidine and/or the histone deacetylation inhibitor trichostatin A induces Rap1GAP expression in thyroid tumor cells, reducing cell invasion and proliferation. Additional studies on these and other novel reagents targeting Rap1 signaling molecules are called for.

Conclusions

Rap1 signaling plays several important roles in tumor cell invasion and metastasis. The full scope of its functions remains unknown; Rap1 can induce very distinct effects depending on the tissue in which Rap1 is expressed. Therefore, the specific functions and effects of Rap1 signaling on metastasis in different tumor types remains a subject of continuing research. Additionally, many proteins contribute to the diversity in the control of tumor invasion and metastasis by Rap1 signaling, and the full panoply of factors that work with Rap1 resulting in diverse control mechanisms is not yet fully elucidated. Future works employing high throughput screening strategies to identify new molecules contributing to Rap1 signaling and real-time monitoring of Rap1 signaling during tumor invasion and metastasis are needed to further define the roles of Rap1.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

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