GTPase Rap1 contribution to tumor development

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The currently available anti-cancer treatments are most effective when a patient is diagnosed with cancer before metastatic formation. To prevent metastatic formation it is important to know the signaling mechanisms that regulate the tumor migration. The small GTPase Rap1 is a molecular switch involved in the metastatic formation of various tumor types. Therefore it is important to understand it signaling mechanism because when its signaling pathway is better understood it might help to develop new anti-cancer treatments. Rap1 is involved in several steps of tumor development which makes it difficult to study and understand its signaling mechanism. For example Rap1 is involved in tumor migration by regulating the cytoskeleton and the activation of integrins and cadherins. Rap1 is also involved in the activation of the MAPK/ERK pathway, regulating cell migration and proliferation. Because Rap1 is involved in several steps of the tumor development it is difficult to find anti-cancer treatments to the effects of Rap1. But there are already some potential drug targets suggested such as JAM-A, an adhesion protein, for breast cancer treatment. A B-Raf kinase inhibitor has been shown to also decrease tumor development and integrin inhibitors have already been studied in preclinical trails as potential anti-cancer drugs. This review will give an overview on what is known about the Rap1 signaling pathways and involvement in tumor development. Finally some potential anti-cancer drug targets will be discussed.

Introduction

The currently available anti-cancer treatments are most effective when a patient is diagnosed with cancer before metastasis development. Therefore it is important to understand the molecular mechanism of tumor migration. One protein which has been shown to be involved in many types of tumor development is the GTPase Rap1. Rap1 is a small-GTPase which can translate extracellular signals to intracellular signals which result in cellular responses including cell proliferation, cell adhesion and migration (1). The activity of Rap1 is regulated by guanine exchange factors and GTPase activating proteins. Activated Rap1 is able to bind to several adaptor proteins which are responsible for various cellular responses. There are two isoforms of Rap1, Rap1a and Rap1b, which differ by only 8 amino acids (2, 3). The two isoforms have been shown to be involved in different signaling pathways but are both involved in the regulation of cadherins and integrins. Integrins regulate the cell to extracellular matrix (ECM) adhesion and cadherins regulate the cell to cell adhesion. The ability of Rap1 to regulate both E-cadherins and integrins suggests that these two pathways are in some way connected to each other (4). Indeed it has been show that Rap1 can link the integrin and cadherin pathways. The dynamic regulation of adhesion sites is important for cell migration and tumor development. Both integrins and cadherins have been shown to be involved in cell migration and tumor development. Tumor development follows different stages and Rap1 has been shown to be involved in different steps depending on the tumor type and environment (1, 5, 6). Because Rap1 is involved in different steps of tumor development, Rap1 might inhibit tumor development at one stage but enhance it in another stage. The activation of Rap1 can have different effects on cellular responses, for example it has been shown that Rap1 activation causes delayed cell spreading and enhanced cell motility in mouse fibroblast cells from C3G knock out mice (1). Rap1 has been shown to be involved in the tumor migration of several cancer types such as breast cancer, prostate cancer, lymphomas, pancreatic cancer, head and neck squamous carcinoma and melanomas.

This ability of Rap1 to regulate different cellular processes makes it difficult to unravel its molecular signaling mechanism. But unraveling the molecular mechanism of Rap1 signaling piece by piece might reveal better anti-cancer therapeutic targets. This could give the patients a higher change of survival. In this review the molecular mechanism of Rap1 activation and the Rap1 involvement in tumor growth and migration will be discussed. Finally some potential drug targets will be discussed.

Rap1 activation

Rap1 is a small G-protein and belongs to the Ras-GTPase family, which can couple extracellular signals to various cellular responses like cell proliferation, cell survival, cell adhesion and cell polarity (7). Rap1 has been shown to be involved in processes such as integrin mediated adhesion and epithelial cadherin (E-cadherin and VE-cadherin)-based adherent junction formation.

Small G-proteins act as molecular switches, they are active in their GTP-bound state and inactive in their GDP-bound state. When Rap1 is in its active GTP-bound state it can bind to several effector proteins, and induces downstream signaling cascades. The exchange of GDP to GTP is catalyzed by

guanine exchange factors (GEFs). The hydrolysis of GTP that alters the signaling, is catalyzed by GTPase activating proteins (GAPs).

Rap1 has a G-domain of 20 kDa and is responsible for nucleotide binding and hydrolysis. This domain has an universal structure and working mechanism for most of the small G-proteins (8). The G-domain has two switch regions and a phosphate binding loop (P-loop). Rap1 shows minor conformational changes in the two switch regions when transitioning from the GDP to GTP-bound state. When GTP is bound to the G-Domain its phosphate forms hydrogen bonds with the main NH groups of residues 32 to 38 in switch I domain and residues 59 to 67 in the switch II domain. When the GTP is hydrolyzed to GDP the γ-phosphate of GTP will be released, the hydrogen bonds will be broken and the two switch regions which where held close together by these hydrogen bonds will relax into a more open conformation (Fig.1).

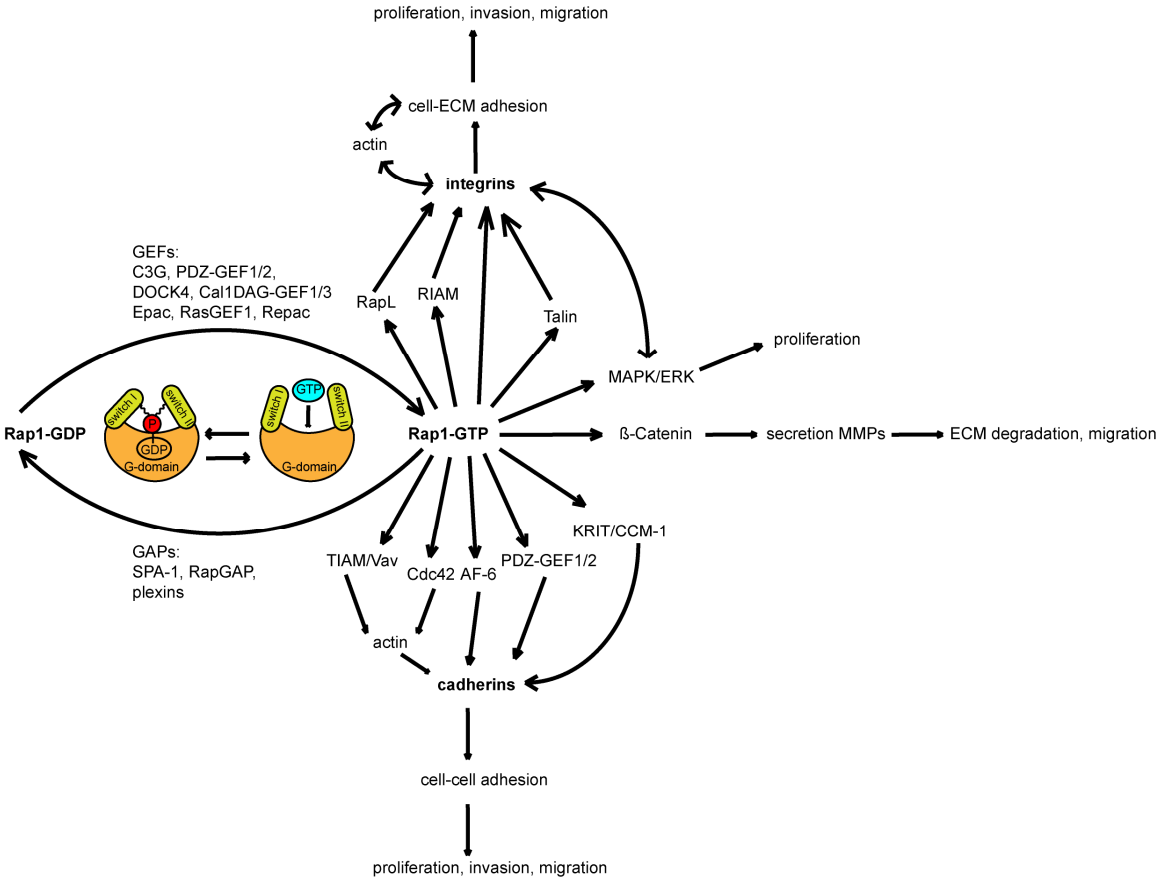

Figure 1: Overview of Rap1 signaling pathways. Rap1 activation is regulated by its GEFs and GAPs. Activated Rap1 can bind and activate several effector proteins. The three major cellular responses of Rap1 activation are cell-cell adhesion or cell-ECM adhesion leading to proliferation, invasion and migration. The third cellular response is the ECM degradation leading to cell migration.

GEFs(8)

GEFs are usually multidomain proteins. Rap1-associating GEFs contain a CDC25 homology domain, mediating the exchange activity, and a Ras exchange motif (REM), stabilizing the CDC25 homology domain. When a GEF binds to Rap1 or other GTPases they can induce a conformational change in the switch regions and p-loop while the remainder of the protein structure remains mostly unaffected. These conformational changes modify the GDP-binding site in such a way that the affinity for GDP is decreased and the exchange of GDP to GTP can occur faster. GEFs interact with switch region 1 and 2, they also insert residues close to or into the Mg^{2+}-binding region and P-loop to induce a conformational change. In the conformation of Rap1, after the conformational change induced by a GEF, the binding of phosphates and Mg^{2+}-ions are inhibited (8). The inhibition of Mg^{2+}-ion binding probably accounts for only a small part of the overall enhancement of GDP release. Because affinity studies show that the P-loop interaction of the G-domain with GDP is the most important for tight binding of GDP. This means that it is probably the

insertion of residues close to or into the P-loop that accounts for most of the enhanced release of GDP.

GEFs have to be activated before they can catalyze the GDP to GTP exchange of GTPases. This activation of GEFs can be regulated by different mechanisms like protein-protein interactions, protein-lipid interactions, the binding of a second messenger like Ca^{2+} or by inducing posttranslational modifications (9). These different regulatory mechanisms can induce GEF translocation to a specific compartment, allosteric changes or displacement of a flanking domain or region. These flanking domains or regions can cover the binding site of the small G-protein and serve as an auto-inhibition mechanism. It is thought that it is not only the location of Rap1 within the cell that is important for its specific activation but also the location of the GEFs that activates Rap1 (10). GEFs that are known to be involved in the activation of Rap1 are Epac1/2, C3G, PDZ-GEF1/2, RasGEF1, CalDAG-GEF1/3, DOCK4 and Repac. The GEFs Epac1 and Epac2 are directly activated by the second messenger cAMP. The cAMP binding site of Epac1/2 is an auto-inhibition region that, as a separate domain, can inhibit the catalytic region of the CDC25 homology domain and blocks the association with Rap1 (11). The binding of cAMP to Epac1 also induces it's translocation to the plasma membrane where it locally activates Rap1 (11). PDZ-GEFs contain a PDZ-domain, and a low affinity cAMP domain that provides an auto-inhibitory function (12). PDZ-GEF1 has been shown to bind scaffold proteins MAGI-1/2 that are in a complex with E-cadherin and β-catenin (13). Immunofluorescense studies together with imunnoprecipitations showed that MAGl localizes to cell-cell contact sites, where it forms a complex with E-cadherin and β-catenin. β-catenin binds to E-cadherin at initial cell-cell contact and recruits the scaffold protein MAGI. The MAGI binds to PDZ-GEF1 which can locally activate Rap1 at cell-cell contact sites. The activation of Rap1 by PDZ-GEF contributes to the maturation of the adherent junctions (14). Using immunofluorescense and electron microscopy *Dube et al*. showed that PDZ-GEF2 depleted cells remained in a zipper like structure indicating that AJ maturation was inhibited. In addition they show that Rap1a and Rab1b have different functions in AJ maturation. Rap1a is involved in the maturation of AJs and Rap1b in the recruitment of E-cadherin at the cell surface of cell-cell contact sites. Although Sakurai *et al*. and Dube *et al*. used two different PDZ-GEFs, together these results indicate that PDZ-GEFs activate Rap1 locally at the plasma membrane at cell-cell contact sites. The locally activated Rap1 recruits more E-cadherin which contributes to the maturation of AJs. The GEF CalDAG-GEF1 has a putative Ca^{2+} and DAG-binding domain its activation can be regulated by either Ca^{2+} -ions, DAG molecules or both (10). The activation of CalDAG-GEF1 is a critical regulator of outside-in activation of Rap1. C3G mediates Rap1 activation induced by receptor tyrosine kinases (RTKs) like Src or preferentially by non-RTKs. C3G binds for example to the Src homology domain (SH3) of Crk and is recruited to nectins at the initial formation of adherent junctions (AJs) (15). The activated C3G can locally activate Rap1 at the site of AJ formation, and there Rap1 will recruit more E-cadherin to mediate the maturation of AJs.

GAPs

To terminate the signaling of small G-proteins bound, GTP should be hydrolyzed. This hydrolysis is catalyzed by GTPase activating proteins (GAPs). Different GAPs use different catalytic mechanism, but there are several factors that make the catalysis effective. These factors are proper orientation and polarization of the attacking water molecule, occlusion of water from the active site and stabilization of the transition state (9). GAPs of Rho, Ran and Ras use a glutamine residue to position the nucleophilic water in a correct way. They also insert an arginine finger into the catalytic site of the GTPases to neutralize the developing negative charge on the leaving group, thereby lowering the activation energy and enhance the reaction rate (16). Because Rap1 does not have a glutamine in its switch domains to position the nucleophilic water together with its GAP, RapGAP uses an asparagine finger in stead of an arginine to insert into the catalytic domain (17). RapGAP has a sequence of 340 aa within its 633 aa which is responsible for its activity. Homologues sequences of this activity domain are found in the human genes SPA1, E6TP1 and TSC2. Similar to GEFs, GAPs can be regulated by protein-protein interactions, protein-lipid interaction, binding of second messengers or posttranslational modifications. These regulatory mechanisms can induce the translocation of GAPs to a specific compartment in the cell, release auto-inhibition by displacement of a flanking domain or region and in some cases allosteric changes in the catalytic domain can occur. GAPs which are known to associate with Rap1 are RapGAP, Spa1 family members which contain a PDZ domain, plexins and E6TP1 (9, 18, 19). Plexins are cell surface receptors and have a specificity for Rap1, they are activated by semaphorins at the plasma membrane. Semaphorins are a family of secreted or transmembrane proteins (20). Dimerization of the plexins is required for activation. Whether semaphorins cause dimerization or that

inactive dimmers exist inactive which are activated by semaphorins is unknown till now.

Rap1 effector proteins

Activated Rap1 can bind to several effector proteins and thereby regulate several cellular mechanisms. For example Rap1 activation can activate integrins or cadherins through binding of several effector proteins. Integrins and cadherins are critical elements in the maintenance of cell architecture and function in developing and adult organisms (21, 22). Integrins regulate the cell to ECM adhesion while cadherins are involved in the regulation of cell to cell adhesions. Both integrins and cadherins have been shown to be involved in tumor development.

Integrin activation

Integrins are heterodimers of non-covalently associated transmembrane type 1 α- and β-subunits (22, 23). Each subunit has a large extracellular domain, a single transmembrane domain and a short cytoplasmic domain. There are in total 24 different integrins, each having a specific combination of α- and β-subunits. These 24 different integrins each bind a defined set of extracellular ligands. The NMR spectra of the αIIb-tail showed a chemical shift when titrated with unlabeled β3-tail. The reverse, thus a chemical shift in the NMR spectra of the β3-tail was observed when unlabeled αIIb-tail was added. This indicates that there is an association between the αIIb-tail and the β3-tail (24). The interaction between the two subunits is composed of hydrophobic and electrostatic interactions. Point mutations in the αIIb-tail that disrupted either hydrophobic or electrostatic interactions destabilized the cytoplasmic complex. The same mutations have been shown to lead to constitutively active integrins suggesting that a conformational change disrupting the cytoplasmic complex leads to activation of the integrins. Two independent studies on the activation upon conformational changes of integrin support the hypothesis that integrins are activated by a conformational change (25). Luo *et al*. showed with cystein crosslinking combined with NMR experiments a specific transmembrane helix contact site between the transmembrane domains of α – and β- subunits. This contact site is lost when the integrin is activated from inside the cell. With EM studies combined with biochemical studies Springer *et al.* showed that integrins have an inactive bended conformation which is changed into a more upright conformation upon activation (26). In this more upright conformation also the transmembrane helix association is disrupted. Thus, integrins are mostly expressed in a low affinity inactive bended state and upon activation a conformational change takes place which converts the integrins in a stretched out high affinity active state (22). Integrins have also been shown to be activated by clustering formation. Li *et al*. showed that there was no difference in affinity to fibrinogen between integrin mutants, where the heterodimerization was affected, and wild type integrins (25). Therefore they suggested that clustering by oligohomomeric association of the transmembrane domains is inducing the activation of integrins. Crystal structures of the αvβ3 integrin suggest that the transmembrane oligohomomeric association is only possible in the active stretched out conformation of integrins. Because when the TM domains are separated in the stretched out conformation the TM domains become available to homomeric interactions (25). Thus, integrins change conformation upon activation from inside the cell and in this activated conformation are able to form clusters.

What is responsible for the activation and conformational change of the integrins? One candidate is talin because talin has been shown to activate the integrin αIIbβ3 by binding to its β3-tail (27). Talin is a major cytoskeleton protein which binds integrins, vinculin and actin filaments, thus talin can also couple the integrins to actin. Talin is 250 kDa and contains a head domain of 50 kDa which is able to bind the β-tail of integrins. This binding can be regulated through Ca1 proteolysis, binding of phosphoinositides or the phosphorylation of talin or integrins (28). NMR studies show that the talin head domain associates with the β-tail of integrins at two regions namely at the membrane proximal region and the N-terminal domain of talin head domain to the c-terminal NPLY region on the β-tail (29). The point mutation Y to A in the NPLY region indeed disrupts the talin binding to the β-tail. In addition biochemical activation studies with purified talin head domain showed a talin concentration dependent integrin activation. When a higher concentration of the talin head domain was added the integrin αIIbβ3 bound more fibrinogen. Thus talin binds to the β-tail especially to β1, β2, and β3-tails thereby inducing a conformational change in the integrins causing the activation of integrins.

Rap1 is also involved in the regulation of integrin activation through inside-out signaling (30). Rap1 activates in particular the integrins which contain a β1, β2 or β3 subunit. When a constitutively active Rap1 mutant Rap1V12 or C3G was expressed in Jurkat cells, more cells bound to ICAM and VCAM compared to control cells (31). Integrin β1 subunit is involved in the binding to VCAM and β2 subunit is responsible for ICAM binding indicating that Rap1 regulates β1 and β2 subunit containing

integrin activation. A recent study on the Rap1 integrin activation mechanism revealed that Rap1 agonist dependent activation of integrins depends on the formation of an activating complex containing the proteins RIAM and talin (32). They used Chinese hamster ovarian cell to reconstruct an integrin activation pathway. In these CHO cells agonist stimulation with PMA need high talin expression and talin binding to β-tail to activate integrins. PMA targets Protein kinase Cα, which is highly expressed in platelets and a key activation mediator of the integrin αIIbβ3. They also show that PMA induces redistribution of talin to the plasma membrane. Rap1GAP almost completely inhibited the PMA induced integrin activation and the expression of a constantly active mutant of Rap1 could activate integrins in the absence of PMA and PKA. These results indicate that Rap1 acts downstream of the PKA. Rap1 co-transfected with talin led to relocalization to the plasma membrane where it forms clusters that co-localize with αIIbβ3 integrins. The ability of talin to bind αIIbβ3 integrins was confirmed by co-immunoprecipitations. In addition RIAM expression showed increased Rap1 induced integrin activation. This was confirmed in a study of Hernandez-Varas *et al*. where RIAM from chemokine-stimulated melanoma cells co-immunopreciptated with talin (33). In RIAM silenced cells talin fails to bind to the β1 subunit. These results suggest that agonist induced Rap1 activation activated integrins in a complex containing RIAM and talin. Rap1 might also regulate integrin activation by its effects on the cytoskeleton. Bertoni *et al*. showed that inhibition of actin polymerization decreased integrin αIIbβ3 activation. The decrease of integrin activity was shown by less fibrinogen binding of megakaroyocytes expressing Rap1V12 in the presence of an actin polymerization inhibitor compared to Rap1V12 expression alone (34). The Rap1 contribution on the actin cytoskeleton dynamics might be through RIAM because RIAM binds proteins of the Ena/Vasp family (4). Proteins of the Ena/VASP family can associate with actin and regulate several cellular processes depending on the actin cytoskeleton like cytoskeleton remodeling, cell polarity, intracellular pathogen motility and lamellipodial and filopodial dynamics in migrating cells. Another possibility is the RIAM involvement in the RhoA activation through inducing Vav2 phosphorylation, which is a GEF for RhoA (33). Activated RhoA activates ROCK which in turn phosphorylates myosin II which then assembles into myosin filaments that associate with the actin cytoskeleton and generate contractile stress fibers needed for cell migration.

Integrins have also been shown to be involved in tumor development in different ways, it can contribute to cell migration, cell proliferation and cell survival (35, 35, 36). Some integrins are promoting while others inhibit tumor development. It has been shown that some integrins can regulate the activation of Src family kinases. The Src kinases can induce activation of other transmembrane receptors such as RTK that regulate cell proliferation and survival. The β3 induced Src activation and not the β1 activation was shown to be important for its transformation properties in colony forming assays (37). This indicates that different integrins have different effects on the tumor progression. Brooks *et al*. showed that the activation of avb3 integrin leads to matrix metalloprotein (MMP)-2 association and release at the plasma membrane (38). MMP proteins are involved in the degradation of the basement membrane and therefore can contribute to the tumor cell invasion and migration. Integrins are also required for cell migration and therefore can also contribute to the formation of metastatic cancers (39). These studies show that integrins can be involved in many different ways in tumor development which is nicely reviewed in a article of R. Rathinan *et al*. (35). Because Rap1 is regulating the activation of some integrins Rap1 might be involved in the regulation of tumor development through the regulation of integrins.

Rap1 activation of Cadherins

Cadherins are single-pass transmembrane glycoproteins involved in homotypic and heterotypic cell-cell adhesion (40). There are several different cadherin types but the type 1 cadherins such as E-cadherin and N-cadherin are the most studied ones (41). Cadherins have an extracellular cadherin domain that in the presence of Ca^{2+}-ions can form dimmers for cell-cell contact. Their cytoplasmic tails connect to the actin cytoskeleton via anchor proteins, called catenins. This connection to the actin cytoskeleton makes the cell-cell adhesion stronger. There are three types of catenins involved in the connection of cadherins to the actin cytoskeleton, α-catenin, β-catenin and p120-catenin. The cytoplasmic tail of cadherin binds to β-catenin which is able to bind to α-catenin. β-catenin has also been shown to be involved in the Wnt signaling pathway. The link to the actin cytoskeleton comes from the α-catenin which binds β-catenin and the actin cytoskeleton. The third catenin p120 also binds directly to the cytoplasmic tail of cadherin but at the membrane proximal-region. The exact function of the p120-catenin is unknown till now but one article showed that when E-cadherin was unable to bind p120 the Rac signaling pathway activation was inhibited (41).

Another study showed in cells which have a very low expression of p120-catenin that the E-cadherin expression at the plasma membrane was less. Therefore they suggested that p120-catenin is responsible for the stabilization of the E-cadherin complex at the plasma membrane (42). When epithelial cells form initially cell-cell contact E-cadherin is recruited to the lateral membrane, at the site of cell-cell contact. This recruitment of E-cadherin is necessary for cell-cell adhesion to mature. When more E-cadherin is recruited at the site of cell-cell contact more E-cadherin dimmers are formed and a zipper like structure arises which develops into a mature linear cell-cell contact. Rap1 has been implicated into two different processes of the maturation of cell-cell adhesion, namely the recruitment of E-cadherin and the regulation of the actin cytoskeleton (43). In the process of E-cadherin recruitment at cell-cell contact sites the GEF, C3G plays an important role in the activation of Rap1. First an initial cell-cell contact is formed either by nectins or by E-cadherins. Nectins activate the tyrosine kinase Src which recruits C3G through Crk and activates Rap1. Rap1 then recruits more E-cadherin to form mature cell-cell adhesions. C3G has also been shown to bind to E-cadherins and activate Rap1 to recruit more E-cadherin (44). This association is only present in immature cell-cell adhesions. Rap1 activity is lost when cell-cell adhesion sites have matured. This means that Rap1 is only involved in the maturation and not in the maintenance of E-cadherin based cell-cell adhesion. The current model of the Rap1 involvement in the maturation of cell-cell adhesion is, first initial cell-cell contacts are formed by nectins and E-cadherins followed by activation of C3G which binds E-cadherin. After activation of Rap1 by C3G more E-cadherin is recruited to the contact site and C3G is displaced by β-catenin which binds PDZ-GEF1 and causes more Rap1 to be activated and recruits E-cadherin to form mature cell-cell adhesions. Rap1 is also involved in the regulation of the actin cytoskeleton by activation through the binding of several of its effector proteins. For example Rap1 can activate Cdc42 through the binding of Vav/TIAM (43, 45). Activated Cdc42 by Vav/TIAM can bind to members of the Wiskot-Aldrich syndrome protein family (WASP). WASP proteins activate the complex of actin related protein 2/3 (Arp2/3), this complex regulates the filamentous actin nucleation and thereby controlling actin cytoskeleton dynamics (46). There are more Rap1 effector proteins that are involved in the maturation of cell-cell contacts namely AF-6 and KRIT-1 (45, 47, 48). AF-6/afadin is activated by Rap1 and binds to several junctional proteins like nectins, ZO-1 and JAM-A and through these proteins also regulates the maturation of AJs. AF-6 also forms a complex with p120-catenin and E-cadherin where it can activate profilins which also regulates the actin cytoskeleton polymerization (43, 48). KRIT-1 is recruited to AJs by activated Rap1 and binds to junctional proteins like talin, β-catenin, p120, ZO-1, and AF-6 which contribute to the maturation of AJs.

Cross talk

Rap1 activation is involved in integrin activation and in the maturation of cadherin based cell-cell contact. This has raised the possibility of Rap1 to link the cadherin and integrin pathway. Indeed it has been shown by Balzac *et al.* that Rap1 links the integrin and cadherin pathways (40). When E-cadherin based cell-cell contacts are disrupted more Rap1 activation takes place. Immunoprecipitation studies showed that Rap1 activation induced by the disruption of cell-cell contact requires Src activation. When cell-cell contacts are disrupted E-cadherin is more redistributed from the plasma membrane to the perinuclear region as has been shown by immunofluorescence studies. In addition to the disruption of cell-cell contact the E-cadherin internalization is required for Rap1 activation. When the E-cadherin endocytosis is blocked by a actin cytoskeleton disruption agent CytD, the activation of Rap1 is reduced upon cell-cell contact disruption. This outside-in activation of Rap1 through E-cadherin turns out to be the signaling pathway that connects the cadherin to the integrin pathway. Because by using a GFP-zyxin as a marker for the stable integrin-mediated adhesion and time lap microscopy video analyses it has been shown that the integrins-mediated adhesion is activated upon the disruption of cell-cell contact. Rap1 has been shown to be activated by the disruption of cell-cell contact thus probably Rap1 links the cadherin pathway to integrin pathway via the outside in activation of Rap1 through E-cadherin endocytosis. In the model for the activation of integrins upon the disruption of E-cadherin involved cell-cell contact, first Rap1 is activated through the disruption of the cell-cell contact en E-cadherin endocytosis. E-cadherin endocytosis activates Src which in turn activates C3G and Rap1. When Rap1 is activated on E-cadherin containing recycling endosomes it can activate integrins through activation of effectors involved in the activation of integrins (Fig.2) (30).

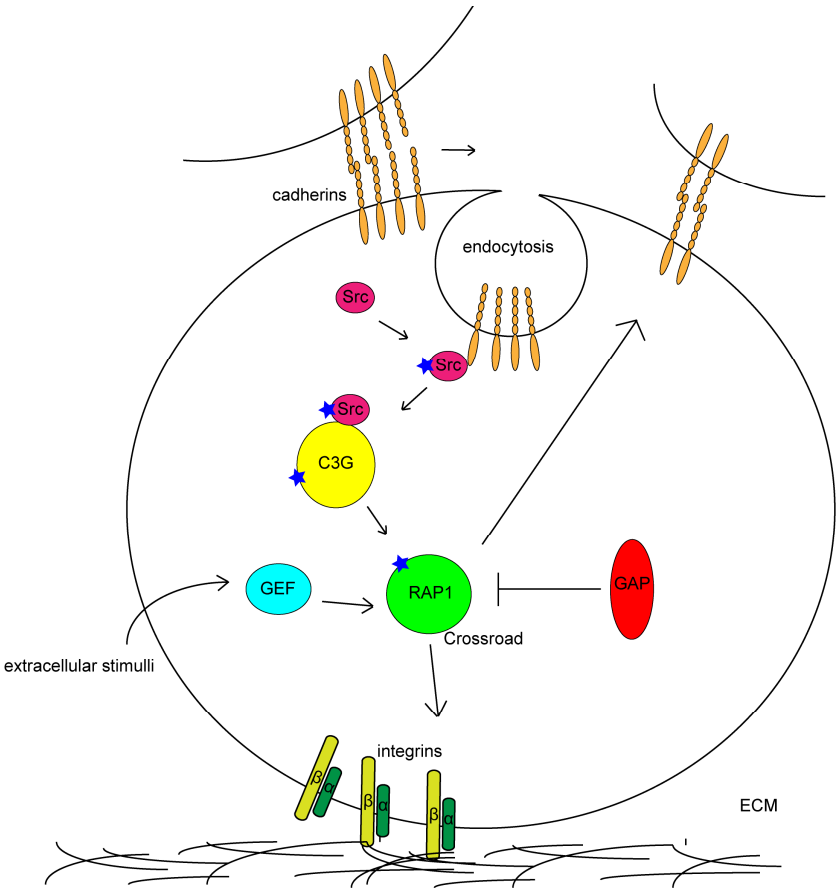

Figure 2: Model of cross talk between integrins and cadherins through Rap1. When cell-cell adhesions are disrupted E-cadherin is endocytosed. E-cadherin endocytosis activates Src which in turn activates C3G and Rap1. When Rap1 is activated on E-cadherin containing recycling endosomes it can activate integrins through activation of effectors involved in the activation of integrins

Tumor migration

Rap1 activation is involved in the regulation of integrins and E-cadherins and can contribute to tumor migration and metastasis depending on the tumor type and environment. There are several steps to be followed by tumor cells to form metastatic cells, Rap1 might regulate different steps depending on the tumor type (49). The first step of metastatic tumor development is the detachment of tumor cells from the primary tumor and becoming invasive. There are different invasive strategies, amoeboid and mesenchymal and both strategies could be affected by Rap1 activity. The mesenchymal invasion strategy consists of 5 steps, in the first step the actin cytoskeleton at the leading edge connects to adaptor proteins and pushes the cell membrane in an outward direction. Proteins involved in this first step are ARP2/3/, WASP, Rac, CDC42, Rho and Ras. Rap1 has been shown to associateVav2/TIAM that activates ARP2/3. The activated Arp2/3 complex can activate WASP, followed by CDC42 activation controlling the actin polymerization. The second step involves the formation of focal adhesions by making contact to the ECM. Next, surface proteases are recruited to the ECM and focalized proteolysis of ECM components takes places. The proteases cleave ECM components such as collagen, fibronectin and laminins and pro-MMPs to create active MMPs that cleave native collagen and other macromolecules of the ECM. This focalized proteolysis of ECM components is needed for the turnover of adhesion sites to be able to migrate. The fourth step involves cell contraction via actinmyosin contraction and the last step is the detachment of the trailing edge.

The amoeboid strategy is characterized by a fast gilding movement which is driven by relatively short and weak interactions with the substrate. The gliding movement is generated by cortical filamentous actin. Mature focal adhesion, stress fibers and focalized proteolytic activity are lacking in the amoeboid invasion. This kind of invasion most commonly occurs in lymphomas and small-cell lung carcinomas, whereas mesenchymal migration mostly occurs in epithelial tumor cells.

The most apparent morphological change cells undergo when they become invasive and follow

the mesenchymal invasion strategy is the epithelial-mesenchymal transition (EMT) where epithelial cells become more fibroblast-like cells (50). The EMT can be induced by different growth factors and involves the downregulation of apical and basoleteral epithelial specific adherent tight junction proteins, reorganization of the cytoskeleton via Rho GTPase proteins and re-expressing mesenchymal molecules such as N-cadherin. When cells are invasive and move through the stroma they have to enter the circulation by penetrating the blood or lymphatic vessels and traffic to other places in the body. When these malignant cells arrest they have to exit the microvasculature, invade into the stroma and finally proliferate to form a metastatic tumor. Rap1 can regulate multiple steps in the metastatic cascade by regulating integrins and cadherins but which steps Rap1 regulates is highly dependent on cell type and the environment. For example activated Rap1 promotes the metastatic invasion of breast, pancreatic and prostate carcinoma cells but inhibits invasion in osteosarcoma and squamous cell carcinoma cells (5).

MAPK signaling pathway

The mitogen activating protein kinase pathway is deregulated in many cancer types. The outside in signaling pathway starts with the activation of receptor tyrosine kinases (RTKs) by ligand binding (51). Ligand binding causes dimerization and phosphorylation of the C-terminal domain. The phosphorylation of the C-terminal domain exposes docking sites for proteins containing a Src homology 2 (SH2) domain or a phosphotyrosine binding (PTB) domain. For example the growth factor-receptor bound protein 2 GRB2 protein contains a PTB domain and binds to the C-terminal domain of RTKs. The binding of GRB2 leads to the recruitment of other effector proteins to the plasma membrane such as SOS that activates RAS proteins that activate cRaf, bRaf and aRaf. The activated Raf proteins phosphorylate tyrosine and threonine residues in MAPK activation loop also known as ERK1/2. Activated ERK1/2 activates proteins like p90 ribosomal S6 kinase (RSK), MAPK-interacting serine/threonine kinase (MNK), and transcription factors leading to various cellular responses including proliferation and integrin activation.

Rap1 has also been shown to influence the MAPK signaling pathway contributing to tumor development. It has for example been shown that Rap1 can regulate the phosphorylation of ERK1/2 independent of NRas or BRaf (52). It might be that Rap1 influences the ERK1/2 phosphorylation through the integrin activation because it has been shown that integrins activation can induce the activation of MAPK.

Breast cancer

From all female cancer diagnosed in Europe 30% is breast cancer. Breast cancer is the leading cause of female cancer deaths. Rap1 activation is involved in the formation of breast cancer tumor metastasis via the regulation of the adhesion protein, junctional adhesion molecule-A (JAM-A) (53). The JAM family of proteins has several important functions such as intercellular junction assembly, cell polarity, cell morphology, platelet activation and leukocyte migration. It has been shown that JAM-A overexpression in breast cancer is associated with a high risk of developing metastasis (54). McSherry *et al.* showed by western blot analysis that JAM-A regulates the protein expression of β1-integrin and the activation of Rap1. They show in addition that tumor migration is dependent on the activity of integrins and Rap1. JAM-A co-imunnoprecipitates with PDZ-GEF2 and AF-6 but not with Rap1 or β1-integrin. This brought them to the hypothetical model of JAM-A signaling cascade in breast tumor cells, were the overexpression of the JAM-A causes more AF-6 and PDZ-GEF2 recruitment to cell-cell adhesion sites. AF-6 and PDZ-GEF2 recruit and activate Rap1 which in turn can activate integrins. Rap1 has been shown to activate the β1-integrin and therefore when more Rap1 is activated more β1-integrin is activated and tumor migration can takes place. Another study on breast cancer cells shows that also *in vivo* enhanced Rap1 activation causes tumor migration of breast cancer cells in nude mice (55). It was suggested that this is caused by the disruption of cell polarity and lumen formation upon Rap1 activation. Thus in breast cancer cells Rap1 activation might regulate the tumor migration in two ways, either through the overexpression of JAM-A resulting in the activation of Rap1 and β1-integrin or alternatively by disrupting the cell polarity and lumen formation.

Pancreatic cancer

Pancreatic cancer patients often die because of the invasive nature of this tumor type. In pancreatic tumors the epidermal growth factor receptor (EGFR) is often deregulated and causes tumor migration (56). Rap1 acts downstream of EGFR and is involved in the tumor migration of pancreatic tumor cells. When Rap1 is downregulated by using siRNA in pancreatic tumor cells the EGFR induced migration is inhibited without influencing the primary tumor growth suggesting that Rap1 activity is required for the EGFR induced tumor migration. EGF binding to EGFR stimulates the activation of Src kinase which phosphorylates CAS. When either Src or CAS are silenced by siRNA the Rap1-GTP loading is decreased and the tumor

migration is reduced in EGF stimulated pancreatic cells. It is known that CAS recruits many adaptor proteins and kinases which are involved in tumor migration. Together these results suggest that when CAS gets phosphorylated it will activate Rap1 in a signaling complex that is formed together with CAS. In fact a signaling model for EGFR induced pancreatic tumor migration was proposed in the study of Huang *et al*. First EGFR is stimulated by EGF binding and activates Src which in turn phosphorylates CAS. The phosphorylated CAS will bind to Nck1 and will regulate RapGEF activation which will activate Rap1. At the end of the cascade activated Rap1 will activate integrins and cadherins mediating the cell invasion and migration (Fig.3).

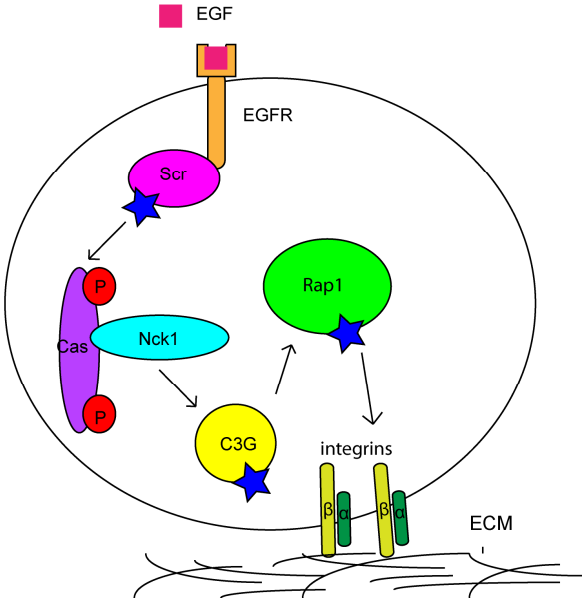

Figure 3: Model of Rap1 activation by EGFR in pancreatic cancer. First EGFR is stimulated by EGF binding and activates Src which in turn phosphorylates CAS. The phosphorylated CAS will bind to Nck1 and will regulate RapGEF activation which will activate Rap1. At the end of the cascade activated Rap1 will activate integrins and cadherins mediating the cell invasion and migration.

Melanoma cancer

The mitogen activating protein kinase (MAPK) pathway is the central pathway in melanoma cancer cells that induces tumor growth and migration. The majority of melanoma cancers cells have activating mutation in N-RAS or BRAF which acts upstream of the ERK/MAPK signaling pathway (57). In addition Zheng *et al.* showed that overexpression of RapGAP, which inactivates Rap1, inhibits the ERK phosphorylation and tumor migration (52). This RapGAP overexpression does not relate to one of the mutations in NRAS or BRAF suggesting that Rap1 regulates ERK phosphorylation independently of N-RAS or BRAF. They showed that RapGAP was downregulated in melanoma cells by promoter methylation. This downregulation of RapGAP increased tumor migration because activated Rap1 could not be inactivated by RapGAP and continued signaling (5, 58). Thus activated Rap1 promotes melanoma cell migration through the activation of ERK/MAPK signaling pathway. In addition it was shown that activated Rap1 activates the $\alpha_v\beta_3$ integrins which promotes melanoma cell invasion. Another study in melanoma cells shows that the adaptor protein of Rap1, RIAM is also involved in the regulation of invasion of the melanoma tumor cells (33). They show that RIAM downregulation by siRNA reduces the beta-integrin-dependent melanoma cell adhesion. In addition they show that the talin and β1-integrin association is lost in RIAM-silenced melanoma cells. These results suggest that the Rap1 dependent activation of RIAM is essential for the activation of integrins via talin. Thus RIAM controls the invasion and growth of melanoma cells through the activation of β1-integrins through the binding of talin. This means that Rap1 can either regulate the integrin activation through the binding of RIAM which forms an integrin activating complex with talin or through the activation of the MAPK pathway which also can activate integrins.

Head and Neck Squamous cell carcinoma (HNSCC)

Rap1 is regulated by several GAPs and in squamous cancer RapGAP activity plays an important role in the tumor migration (59). When RapGAP is expressed it has been shown to delay the cell transition form the G1 phase to the S phase (58). Thus when Rap1 is active it promotes the cell transition from G1 to S phase and therefore also tumor growth. This effect of Rap1 on the cell cycle is probably indirect because it has been shown for integrins that they can mediate the transitions in the cell cycle (36). Thus when Rap1 activation is inhibited by RapGAP the activation of integrins is inhibited and cell transition from G1 to S phase does not occur. The tumor growth suppressor RapGAP has been shown to induce the secretion of metalloproteinase 9 (MMP9) which is involved in the degradation of the ECM (60). MMPs are zinc-dependent proteolytic enzymes that induce invasion and tumor progression by destroying the basement membrane. The remodeling of the ECM is required for cell migration and therefore also involved in the formation of tumor metastasis of many tumors. The gelatinases MMP2 and MMP9 degrade collagen type 5 which is a key step in the formation of tumor metastasis from Squamous cell carcinoma (SCC). When more RapGAP is expressed in SCC cells more MMP9 gets secreted which promotes tumor cell migration. Although RapGAP inhibits tumor growth it promotes the formation of metastasis by inducing secretion of MMP9 an MMP2. This is a nice example of Rap1 acting on different levels of tumor migration. When active in HNSCC it enhances tumor growth but inhibits tumor invasion through MMP secretion. In contrast with an other study on HNSCC cells, activated Rap1 enhanced tumor invasion through the secretion of another MMP, the MMP7 (61). This enhanced secretion was probably induced by the enhanced transcription of MMP7 induced by β-catenin. It was suggested that this enhanced transcription of MMP7 is regulated by activated Rap1 that translocates β-catenin form the cytosol to the nucleus (59)(59, 62, 63). When β-catenin translocates from the cytoplasm to the nucleus it triggers gene transcription of for example MMP7. β-catenin is also a central molecule in the Wnt signaling pathway which has been shown to be involved in tumor growth and invasion. The enhancement of β-catenin-mediated MMP7 transcription also increased the invasiveness of SCC cells. In fact immunohistochemical studies showed that high β-catenin together with high concentration of Rap1 are associated with a more advanced N-stage, resulting in the patient developing lymph node metastasis.

Thus Rap1 is involved in different steps of SCC tumor development on one hand it promotes the cell cycle and on the other hand it regulates the invasion by regulating the β-catenin translocation and MMP2/7/9 secretion (Fig.4).

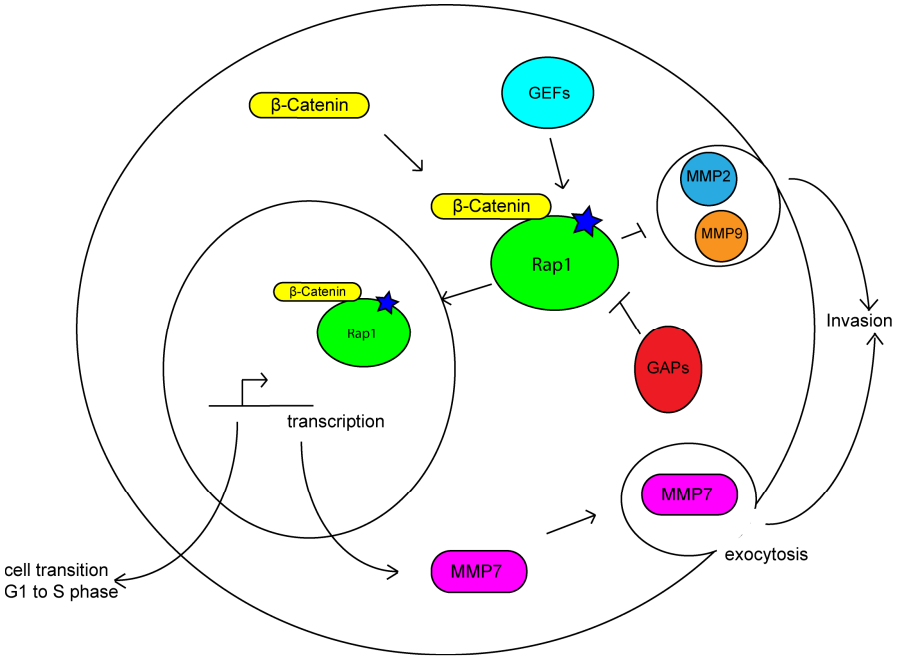

Figure 4: Model of activated Rap1 regulating MMP activation and release in HNSCC. Activated Rap1 translocates β-catenin from cytosol to nucleus, where the β-catenin is a cofactor for the transcription. The translocation of β-catenin causes enhanced transcription of MMP7. MMP7 degrades the basement membrane and induces invasiveness of the tumor cells. In contrast activated Rap1 inhibits the release of MMP2 and MMP9 which causes inhibition of the invasion of tumor cells.

Neuroendocrine tumors of the digestive tract

Neuroendocine tumors of the digestive tract (dNETs) are derived from either the pancreas or the gut. This type of tumor cells does not involve mutations in classical oncogenes like Ras, Myc, Fos, Jun or in tumor suppressors like p53. The overexpression of EGFR which occurs in many types of tumors is also not overexpressed in dNETs (64). The MAPK signaling pathway induces proliferation and protection from apoptosis MAPK is activated by B-Raf and Raf-1 which are stimulated by RAP1 (Fig.5) (57) (2, 64). The continuously active mutant of B-Raf appears in malignant melanomas and also in thyroid carcinomas suggesting that B-Raf is a major oncogene in these tumors. Therefore the Rap1 involvement in the B-Raf activation in dNETs was also determined. Rap1 and B-Raf are expressed in high levels in most dNETs which suggest that they play a role in the tumor development. It was found that the expression of Rap1 together with B-Raf is sufficient to activate MAPK and induce proliferation. When a Raf kinase inhibitor BAY43-9006 was added the proliferation and surviving signaling through MAPK was inhibited. This result suggests that Raf kinase inhibitors might be used as potential anticancer drugs to treat patients with dNETs.

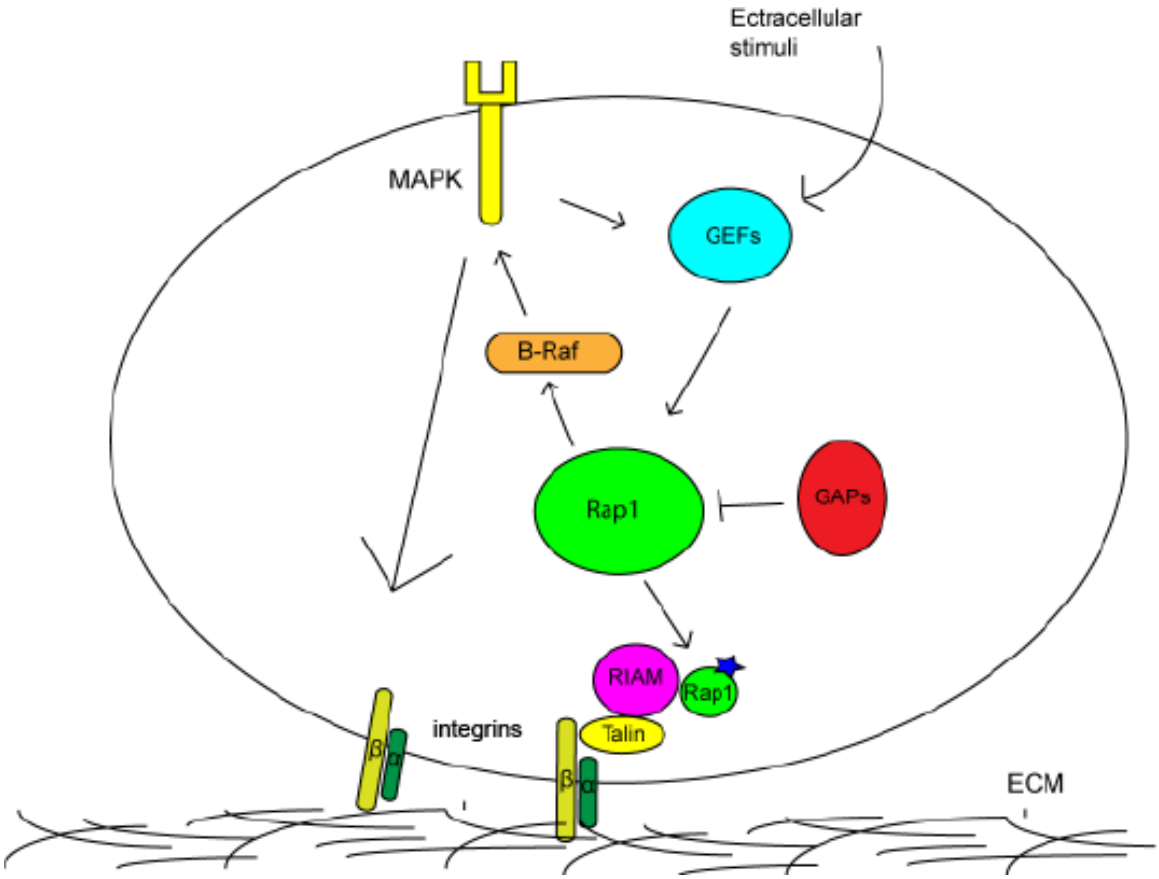

Figure 5: Model for Rap1 signaling in dNETs. Both Rap1 and B-Raf are high expressed in dNETs. Rap1 can activate B-Raf which in turn will activate MAPK. Activated MAPK will activate integrins which can cause cell migration and proliferation. Rap1 can also directly activate integrins by the binding of RIAM which binds talin. Talin will bind and activate integrins causing proliferation and migration.

Colorectal cancer

Colorectal cancer (CRC) is a malignant cancer and expresses reduced E-cadherin, which has been linked to increased invasiveness in several metastatic formations (65). The transfection with siE-cadherin in CRC cells induced more tumor growth compared to the control transfection with siLuciferase. The tumor invasion was also increased upon transfection of CRC cells with siE-cadherin which suggests that E-cadherin expression reduces cell invasion. E-cadherin associates with β-catenin and when E-cadherin was knocked down the nuclear levels of β-catenin increased. This suggest that E-cadherin regulates the transcription by binding the free β-catenin in the cytosol preventing its translocation to the nucleus and thereby reduces transcription efficiency. This is in agreement with what was found in HNSCC where activated Rap1 increased transcription of MMP7 when more β-catenin was translocated to the nucleus. These results suggest that E-cadherin binds β-catenin and reduces β-catenin translocation to the nucleus and in many tumor cells E-cadherin expression is reduced causing enhanced translocation of β-catenin and invasion. In agreement with this they also found N-cadherin which is a mesenchymal marker overexpressed after siE-cadherin transfection. The knock down of E-cadherin also caused a change in cell shape which indicated a EMT transition of the CRC cell which might induce migration. Thus the knock down of E-cadherin regulates the tumor growth and migration of colorectal cancer by regulating the β-catenin translocation to the nucleus and thereby the transcription efficiency.

Leukemia

Rap1 activates the Ras/ERK signaling pathway in T-cells which induces proliferation (3). When Rap1 activation is deregulated the continuous T-cell proliferation can cause the development of leukemia. The prenylation of Rap1 is important for its membrane association and thus proper localization. Statins have been shown to reduce the prenylation of Rap1 and causes the mislocalization of Rap1. Statins are hypocholesterolaemic drugs, initially used to lower cholesterol levels to threat

patient with cardiovascular diseases. Later it has been shown that statins also have an effect on the immune system (66). Statins reduce the prenylation of Rap1 by inhibiting the reductase HMG-coA which results in a decrease in melvoic acid. Melvoic acid is an upstream substrate for the formation of cholesterol, farnesyl and geranylgeranyl isoprenoids. These isoprenoids are added posttranslationally to the carboxy terminal cysteine of CAAX motifs on proteins, and act as anchoring for proteins to membranes. Statins reduce the prenylation of Rap1 which causes mislocalization and as a consequence less activation of the LFA-1 integrin, less interaction with ECM protein ICAM-1 and no transendothelial migration (TEM). Because of these consequences of Rap1 mislocalization tumor development is inhibited. It was suggested that that the prenylation of Rab1b in particular is regulated by statins, since Rap1b but not Rap1a depletion causes similar effects as statins. Therefore it was suggested that Rap1b activates the integrin LFA-1 and the adhesion to ICAM-1 by proper localization caused by prenylation. Rap1b stimulates the tumor growth and migration through the activation of the integrin LFA-1.

B-cell lymphoma

B-cell lymphomas are tumors derived from transformed B-cells which have left the circulation and formed tumors in different organs. It has been shown that transformed B-cells leave the circulation mediated by the same chemokine and integrin mediated mechanism as normal B-cells (67). Rap1 regulates the cytoskeleton and integrin activation, which are involved in the chemokine induced adhesion and migration of B-lymphoma cell lines. In A20 murine B-lymphoma cell line it was shown that Rap1 activation regulates the entering of tissue and formation of tumors by B-cells which have left the circulation. *In vitro* assays with A20 murine B-lymphoma cell lines show that Rap1 activation is required for the A20 cells to efficiently adhere to vascular endothelial cells, and undergo the transendothelial migration. Thus in B-cell lymphomas Rap1 activity is required for the formation of tumors in several organs within the body.

Anticancer therapies

Rap1 activation is associated with several steps in tumor development and its activation can also be reduced by different strategies at different steps of the tumor development process. Rap1 activity is regulated by different GEFs and GAPs and activated Rap1 binds different effector proteins which induce cellular responses such as adhesion, migration and proliferation. Therefore Rap1 itself is a potential drug target but also its activators and effector proteins can be potential drug targets. JAM-A has been shown to regulate Rap1 activation in breast cancer cells and regulates the tumor growth and migration. In a study were JAM-A was downregulated by miR-145 a reduced tumor invasion and migration was observed suggesting a potential role for JAM-A inhibition in an anti- tumor cell migration therapy (54). Neuroendocrine tumor development occurs via the MAPK signaling pathway activated by the oncogene B-Raf. B-Raf can be activated by Rap1 therefore the inhibition of Rap1 or B-Raf might inhibit tumor development. It has been shown that a B-Raf kinase inhibitor inhibited the development of dNETs which suggest that the kinase inhibitor can be a potential anti-cancer drug (64). In T-cells, statins have been shown to inhibit the prenylation of Rap1 and causes misclocalization of Rap1. The mislocalization of Rap1 inhibited the activation of integrin LFA-1 and thereby contributed to a reduced tumor development (3). Two studies on different tumor types showed that the translocation of β-catenin from the cytosol to the nucleus causes more tumor migration (61, 65). This enhanced tumor migration is probably an affect of β-catenin mediated transcription of MMP7. The inhibition of β-catenin translocation might be a potential strategy to treat several tumor types and prevent tumor growth and migration. MMP enzyme proteins degrade the basement membrane and increase tumor migration. Thus MMP proteins regulated by the activation of Rap1 might also be potential drug targets to decrease the invasion and migration of tumor cells. B-cell and T-cell invasion to develop leukemia or B-celllypmhoma are highly dependent on the integrin mediated adhesion (3, 3). When Rap1 activation is inhibited the formation of the integrin activating RIAM/talin complex might be inhibited which will reduce the activation of integrins and tumor migration. The individual proteins of the activating protein complex, RIAM and talin might also be potential drug targets especially for B-cell lymphomas and leukemia. Integrin adhesion to the ECM is important for various tumor types and tumor migration and therefore might be potential drug targets in the treatment of several cancer types. Cadherin mediated cell-cell adhesion is also important for tumor cell migration. Rap1 induces the activation of E-cadherin which is a tumor suppressor and therefore the overexpression or more activation of Rap1 locally at cell-cell adhesion sites might reduce tumor migration. The location of the activated Rap1 in this case is important because when more activated Rap1 is present near integrins it might induce the invasion and migration. It was already shown that the location of Rap1 as well as its GEFs

and GAPs are important for its localized activation. Thus individual proteins in the Rap1 pathway or Rap1 itself might be potential drug targets but probably it is tumor type specific which protein is the best target.

Concluding remarks

Today still a lot of patients diagnosed with cancer die form this disease especially when the patient develops metastasis. The currently available drugs are most effective when a patient is treated before metastatic formation. The small Ras-GTPase Rap1 is involved in the development of many tumor types and is a frequently studied protein (33, 52, 53, 55, 57-59, 61, 64). Rap1 is involved in different stages of tumor development because it can regulate different steps during tumor development depending on the tumor type and environment. Rap1 act as a molecular switch with an active GTP-bound state and an inactive GDP-bound state. The activation of Rap1 is regulated by GEFs which catalyze the GDP to GTP exchange. The signaling of activated Rap1 is altered by GAPs which catalyze the GTP hydrolysis. GEFs and GAPs in turn are regulated by other proteins (9). When Rap1 is activated it can bind to several effector proteins such as RIAM, AF-6 and KRIT-1/CCM. Through these effector proteins Rap1 regulates the cytoskeleton dynamics, integrin and cadherin activation which are all involved in tumor development and migration. Rap1 activates integrins by activating RIAM which binds to talin. The talin head domain can activate integrins by binding to the β-integrin tail and induce a conformational change (4). Activated integrins regulate the cell to ECM adhesion and is shown to be involved in tumor development. This means that Rap1 activation might be regulating tumor migration by regulating the integrin activation. Rap1 can also activate E-cadherin which is involved in the cell to cell adhesion and in tumor cell migration. Locally activated Rap1 at cell-cell interaction sites recruits more E-cadherin to regulate the maturation of cell-cell adhesion and regulate cell migration. The third way where Rap1 can regulate cell migration is by regulating the cytoskeleton dynamics (43, 45). Rap1 can activate Cdc42 through the binding of Vav/TIAM. Activated Cdc42 can activate WASP which activates the Arp2/3 complex controlling the actin polymerization. Alternatively Rap1 can bind AF-6 which binds profiling, which is another protein regulating actin polymerization (45, 48). Because Rap1 can regulate cell migration through the activation of integrins, cadherins or the actin cytoskeleton polymerization, it can be involved in tumor migration via different signaling pathways. The Rap1 involvement in tumor migration is dependent on the tumor cell and environment. In many cancer types Rap1 is involved in the activation of integrins for example in breast cancer, pancreatic cancer, melanoma cancer, HNSCC, leukemia and B-cell lymphoma. In breast cancer cells the junctional adhesion protein JAM-A activates Rap1 and increases the integrin expression (53, 54). JAM-A recruits PDZ-GEF2 and AF-6 to cell-cell adhesion sites which activate Rap1. Rap1 activates the integrins which will form cell to ECM adhesion sites. In breast cancer it has been shown that JAM-A is overexpressed and can lead to enhanced tumor migration through increased activation of integrins by activated Rap1. The enhanced migration could also be induced by the enhanced activation of MMPs. Because it has been shown that integrins can also activate MMPs to degrade the basement membrane and induce tumor invasion and migration (35, 38). In HNSCC MMP activation through the activation of Rap1 is important for its invasive nature. It was suggested that in this tumor activated Rap1 chaperones β-catenin from the cytosol to the nucleus where β-catenin act as a cofactor for transcription of MMP7. When more MMP7 is expressed and released from the cell it will degrade the basement membrane and thereby contribute to tumor invasion and migration. In contrast they show in the same study that activated Rap1 inhibits the release of MMP2/9 and tumor cell invasion. Thus activated Rap1 enhances tumor invasion by the enhanced transcription of MMP7 but inhibits tumor invasion through the decreased activation of MMP2/9. How the regulation of MMP activation by activated Rap1 exactly occurs is yet unknown. The enhanced tumor migration by the translocation of β-catenin from the cytosol to the nucleus is in agreement with a study in CRC cells where the downregulation of E-cadherin induces more β-catenin translocation and tumor migration (65). It might be that when E-cadherin is at cell-cell contact sites it recruits the free β-catenin at the plasma membrane and as a consequence β-catenin can not be translocated to the nucleus and less transcription will take place. In this way E-cadherin acts as a tumor suppressor. Rap1 can activate the MAPK signaling pathway probably through the activation of integrins. It has been shown that integrins can activate MAPK via several pathways. In dNET cells it has been shown that Rap1 activates MAPK via the activation of B-Raf and Raf-1 (2, 64). The activation of the MAPK pathway induces cell proliferation and cell migration. In contrast it has been shown in melanoma cancer cells that Rap1 activates MAPK independent from B-Raf and Raf-1 (57). Thus it might be that Rap1 activates integrins which are able to activate MAPK through the activation of B-Raf or Raf-1. The

activation of MAPK is involved in tumor growth and migration.

Rap1 is involved in many different tumor types and regulates different steps of the tumor development. Therefore it is difficult to find suitable anti-cancer treatments against Rap1. There are several therapeutic options to inhibit Rap1 signaling either by direct inhibition of Rap1 itself or by inhibiting or compensating for Rap1 regulators or effector proteins. For example the overexpression of JAM-A in breast cancer cells causes enhanced Rap1 activation and thereby promote tumor migration (53). Therefore JAM-A might be a potential anti-migratory target. The ERK/MAPK signaling pathway which is also regulated by the Rap1 activation regulates tumor growth and migration. Therefore the ERK/MAPK signaling pathway might also be a potential anti-cancer therapeutic target, either by regulating the activation of Rap1 directly or by one of the downstream effector proteins. In T-cells statins have been shown to inhibit Rap1 prenylation which resulted in a decrease of tumor development. This suggests that statins might also be a potential anti-cancer drugs (3). Statins are already used in the clinic to treat patient with cardiovascular diseases and it has already been shown to decrease tumor development. Therefore statins are very attractive to use as anti-cancer treatment in various cancer types. Rap1 regulates the activation of integrins which contribute to tumor growth and migration therefore they might also be potential therapeutic targets. There are already inhibitors of integrins in the clinical trails for the treatment of cancer(35). Rap1 integrin activation might be inhibited by the inhibition of RIAM binding to talin or the inhibition of talin binding to β-integrin tails. This might give a more specific Rap1 induced inhibition and therefore might be more tumor specific and causes less side effects. E-cadherin acts as a tumor suppressor and can be recruited to the plasma membrane by activated Rap1. A anti-cancer treatment strategy might be to locally activate Rap1 at the plasma membrane at cell-cell adhesion sites. Then more E-cadherin will be recruited less β-catenin can translocate to the nucleus and this will inhibit the tumor development. The challenge of developing anticancer therapeutics is to only attack the tumor cells and not the healthy cells therefore a lot of research is still needed to explore the Rap1 signaling mechanisms and contribution to tumor development. When Rap1 signaling mechanisms are better understood it might be possible to develop anti-cancer therapeutics although in this case because of the many different roles of Rap1 activation the therapeutics probably should be adapted to tumor type.

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