p120 catenin: an essential regulator of cadherin stability, adhesion-induced signaling, and cancer progression

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Summary

p120 catenin is the better studied member of a subfamily of proteins that associate with the cadherin juxtamembrane domain to suppress cadherin endocytosis. p120 also recruits the minus ends of microtubules to the cadherin complex leading to junction maturation. In addition, p120 regulates the activity of Rho family GTPases through multiple interactions with Rho GEFs, GAPs, Rho GTPases, and their effectors. Nuclear signaling is affected by the interaction of p120 with Kaiso, which regulates Wnt-responsive genes, as well as transcriptional repression of methylated promoters. Multiple alternative spliced p120 isoforms and complex phosphorylation events affect these p120 functions. In cancer, reduced p120 expression correlates with reduced E-cadherin function and tumor progression. In contrast, in tumor cells that have lost E-cadherin expression p120 promotes cell invasion and anchorage-independent growth. Furthermore, p120 is required for Src induced oncogenic transformation and provides a potential target for future therapeutic interventions.

Keywords

RhoA; Rac1; cell-cell junctions; Kaiso; EMT; E-cadherin; microtubules; endocytosis; Src; RTK

1. Introduction - History of p120

The p120 catenin (p120ctn; p120 herein) protein was originally identified in a screen for substrates of the Src tyrosine kinase as a 120kDa protein whose phosphorylation on tyrosine residues correlated with cellular transformation ([1], reviewed in [2]). A number of additional Src substrates, including Focal Adhesion Kinase (FAK), p130cas, talin and cortactin were also identified in the screen and subsequently shown to regulate cytoskeletal remodeling and focal adhesion signaling [3]. Unlike the other Src substrates, p120 was shown to interact with classical cadherins at the adherens junctions (AJs) [4–6], where it suppresses cadherin endocytosis [7–9]. p120 regulates cytoskeletal re-organization by affecting the activities of Rho GTPases [10–12], or by mediating the association of microtubules to the cell junctions [13–16]. Additionally, p120 interacts with the transcription factor Kaiso to regulate nuclear signaling events [17, 18]. Recent studies have re-evaluated

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the role of p120 in cellular transformation, indicating that p120 is required for the anchorage-independent growth of tumor cells lacking E-cadherin, or of cells overexpressing Src [19, 20].

2. Structure

2.1 Family members

p120 is the better studied member of a subfamily of armadillo repeat containing proteins, that share a common genetic and protein structure; the other members are also found at cell-cell junctions and can participate in Rho signaling [2]: ARVCF and δ-catenin are found at AJs, like p120; plakophilins at desmosomes; and p0071 at both (reviewed in [21–23]). p120 is ubiquitously expressed, while the expression of other family members is more restricted, suggesting specialized cellular functions. One difference between p120 and the other three family members found at AJs is that p120 lacks a C-terminal PDZ binding motif with high affinity for Erbin and hScrib [24, 25].

p120 is also superficially similar to β-catenin, both in structure, being a member of the armadillo repeat (ARM) family of proteins, and in function, interacting with cadherins at AJs and regulating transcription. p120's functions however, are substantially different from those of β-catenin [2]. p120 and β-catenin associate with the cadherin juxtamembrane (JMD) and the catenin binding domains (CBD), respectively. Surprisingly, the mechanism is similar, with both proteins utilizing basic grooves within their ARM domains to recognize charged and hydrophobic residues in the JMD or CBD [26]. A distinct set of surface exposed residues is responsible for the differential ability of these proteins to bind either the JMD or the CBD. Through it's binding to the JMD, p120 selectively regulates cadherin endocytosis and stability. Another important difference is that p120 has a well-established role in regulating members of the Rho family of small GTPases, which are key mediators of cytoskeletal dynamics and cadherin-mediated cell-cell adhesion (reviewed in [27]).

2.2. Isoforms

Cloning and analysis of the p120 gene revealed 4 alternately spliced exons and 4 transcriptional start sites that together can potentially produce 64 different isoforms of p120 ([28, 29], reviewed in [21, 30]. p120 isoforms are named after the transcriptional start site used (1–4), and the alternatively spliced exons they express (A–D). The complexity of p120's exon-intron structure and regulation and potential competition for cadherin binding from other family members [31, 32] suggest that p120 and the JMD play a key regulatory function in the cadherin complex. This hypothesis is further supported by evidence that p120 can be phosphorylated in multiple serine, threonine and tyrosine residues that affect its function [33–36]. The potential of this variation in protein sequence and phosphorylation status makes p120 an interesting but also a complex member of AJs.

The functions of particular p120 isoforms are largely unexplored. However, a change in the ratio of p120 isoforms is evident in epithelial vs. mesenchymal cells [5, 29, 37]. During epithelial-to-mesenchymal transition (EMT), or after ectopic Snail expression, the larger

p120 isoform 1 is induced [38–40] via a process that involves Epithelial Splicing Regulatory Proteins 1 and 2 [41].

2.3 Evolution

Analysis of proteomes from 14 different vertebrate and metazoan species shows that the p120 protein family evolved from a common δ-catenin-like ancestor present in all metazoans [42, 43]. Successive rounds of gene duplication and diversification are responsible for the seven p120 family members present in the human genome. Ablation of the single δ-catenin-like p120 family member in invertebrate species (e.g., *Droshophila melanogaster*, *Caenorhabditis elegans*) produced minor adhesion phenotypes and did not affect survival [44–46]. In contrast, p120 knockdown or depletion in vertebrates is embryonic lethal [47–51]. Paradoxically, p120 has evolved in vertebrates into a ubiquitously expressed protein that is essential for life, whereas δ-catenin, ARVCF and p0071 are more restricted in expression and their loss better tolerated.

3. Function

3.1 Cadherin stability and function

It is well established that p120 binding to the JMD of the cadherin cytoplasmic tail is directly responsible for stabilizing cadherin expression at the cell surface, as well as for inducing cadherin clustering that results in the formation of AJs (reviewed in [21, 52]). The dissociation of p120 from the cadherin complex results in endocytic internalization of cadherins [8, 9, 53] (Figure 1). Endocytosed cadherins can be either degraded in the lysosomes, or they can be recycled to the plasma membrane via a process that may involve p120 binding and the Rap1 GTPase [53, 54]. Thus p120 is a master regulator (a rheostat) that controls cadherin stabilization and assembly into AJs.

Insight into the mechanism by which p120 binding prevents cadherin endocytosis was provided recently by the elucidation of the p120 structure in association to E-cadherin [26]. Both "dynamic" and "static" interactions contribute to the binding of p120's ARM domain with the cadherin JMD. Importantly, these interactions include JMD residues that are implicated in clathrin-mediated endocytosis and Hakai-dependent ubiquitination of E-cadherin [26] (Figure 1). Based on the structural evidence, p120 binding physically impedes the interaction of cadherin molecules with either E3 ligases or the endocytic machinery.

Interestingly, the N-terminal domain of p120 is in close proximity to the CBD and β-catenin. This p120 domain is highly regulated by alternative splicing and by phosphorylation. Serine/ threonine phosphorylation of p120's N-terminus is thought to control E-cadherin dynamics at the cell membrane [35]. The data argue that both cadherin binding, which is mediated by p120's central ARM domain, and signaling events mediated by p120's N-terminus are required for cadherin stabilization.

Surprisingly, early studies argued that the JMD has both positive and negative functions in cadherin clustering and cell-cell adhesion (reviewed in [21]). A model whereby p120 is "activated" upon cadherin *trans* dimerization was proposed, whereas intracellular signaling events were suspected to "de-activate" p120 and block its function in promoting cell

adhesion [21]. This model was supported by the observation that cadherin activating antibodies induce cadherin conformational changes and promote cell-cell adhesion via the de-phosphorylation of selective p120 residues in the N-terminus [55]. The positive effect of dephosphorylated "activated" p120 may be due to increased cadherin clustering, possibly via direct p120 dimerization [26]. Alternatively, p120 may serve as a scaffold for additional proteins, enabling them to directly affect adhesion or to regulate the function of other cadherin/catenin complex proteins. One candidate is PLEKHA7, a novel p120 N-terminal interacting protein that is required for mature junction formation [16, 56]. Additionally, p120 can recruit a number of kinases and phosphatases that could influence adhesion by affecting the entire cadherin-catenin complex. p120 "de-activation" may also relate to signaling events leading to cadherin endocytosis. Recently, p120 was shown to mediate the interaction of E-cadherin complexes with a protein called Numb. Numb is normally phosphorylated by aPKC, a main component of the tight junctions. However, upon dephosphorylation, Numb can bind to either the C-terminus of p120 or to non-p120-bound E-cadherin, mediating in both cases clathrin-dependent endocytosis of E-cadherin [57] (Figure 1).

Finally, the signaling that regulates the interaction of p120 with cadherins likely includes dynamic phosphorylation of p120 and cadherin (reviewed in [58]). Tyrosine phosphorylation of E-cadherin at Y755/756 or VE-cadherin at Y658 disrupts p120 binding [26, 59, 60] (Figure 1). Additional kinases, like PAK (p21 Associated Kinase) or CK1 epsilon can reportedly also phosphorylate cadherins and disrupt p120 association [61, 62]. Clearly, the JMD plays a critical role in cadherin stability and cell adhesion, and its function is highly regulated by phosphorylation and by interaction with a host of p120-related proteins (including isoforms and family members).

3.2 Regulation of Rho GTPase signaling

The Rho GTPases RhoA, Rac1, and Cdc42, are molecular switches that regulate cell migration, as well as cadherin-mediated adhesion and cell-cell junction formation through the manipulation of the actin cytoskeleton [63–67]. Interestingly, cadherin ligation can reciprocally modulate the signaling of Rho GTPases, and such regulation is thought to require p120 (reviewed in [27]). Experiments to date suggest the existence of several different mechanisms by which p120 can affect Rho GTPases:

1. The first involves the direct association of p120 with Rho GTPases. When not bound to cadherins, p120 acts as a guanine-nucleotide dissociation inhibitor (GDI) and directly binds to and suppresses RhoA activity [36, 68]. At least two p120 domains are essential for a stable interaction with RhoA and inhibition of its activity; a central polybasic region termed "αRho" (amino acids 622-628) [36, 69] and an N-terminal region (including amino acids 131-156) [70]. The interaction of p120 with RhoA is further stabilized by tyrosine phosphorylation of p120 at Y217 and Y228 by Src, or destabilized by phosphorylation at Y112 by Fyn [36]. In addition to RhoA, p120 can also associate with Rac1 and especially, Rac1b, an alternatively spliced constitutively active Rac1 isoform. This interaction, however, does not interfere with the intrinsic GTPase activity of either Rac1 or Rac1b [71].

2.　　　The second mechanism involves the association of p120 with Rho GEFs or GAPs, proteins that regulate the activation or inactivation of downstream Rho GTPases. At nascent AJs, cadherin-associated p120 interacts with Vav2 locally to activate Rac1 and Cdc42 to promote reorganization of the actin cytoskeleton [11, 50]. It is now clear that association with Rho regulatory proteins in addition to Rho GTPases is a general feature of the p120 family of proteins. The p120 family member p0071 has been shown to associate with the Rho GEF Ect-2 to regulate cytokinesis [72], while δ-catenin binds p190 RhoGEF [73]. p120 is also responsible for the cortical localization of p190 RhoGAP, a negative regulator of RhoA, which is activated by Src signaling and mediates Rac-mediated suppression of RhoA [74].

3.　　　A third mechanism of action is p120 interaction with Rho GTPase effector proteins. p120 can associate with and recruit Rho-associated protein kinase 1 (ROCK1) to sites of cell-cell contact [75]. Additionally, cytosolic p120 associates directly with and inhibits the activity of myosin phosphatase Rho–interacting protein (Mrip), an antagonist of Rho/Rock function and ROCK activation [76]. While questions remain, the data suggest that p120 family members act as scaffolds to promote the association of positive or negative regulators with Rho GTPases and their subsequent effector proteins at particular subcellular sites depending on contextual signals. Consistent with this hypothesis, the recruitment of Rac1 to newly formed cell-cell contacts and the accumulation of the Rac1 effector PAK to these sites require the cadherin JMD and p120 association [77].

Complicating matters, 1) p120 isoforms differ in their ability to regulate Rho GTPases. Expression of the mesenchymal p120 isoform 1 inhibits Rho activity, while expression of the epithelial p120 isoforms 3 and 4, has no effect, or activates RhoA, respectively; 2) Since the stability of microtubules can impact the activities of Rho GTPases via microtubule-binding proteins such as GEF-H1, the ability of p120 to associate with and stabilize microtubules can indirectly influence Rho GTPases; and 3) The effects of p120 on Rho GTPase activation/inhibition may depend on cell context, and specifically the type of cadherins that a cell expresses.

3.3 Nuclear signaling

Another signaling function of p120 is the regulation of gene transcription, particularly the convergent regulation of canonical Wnt/β-catenin target genes such as Cyclin D1 and Wnt-11. Regulation of transcription by p120 occurs through its interaction with the transcription factor Kaiso (reviewed in [78, 79]). Kaiso recognizes two DNA sites: a sequence-specific DNA consensus site (5′CTGCNA3′) [80] and methylated CpG dinucleotides [18, 80]. Many Wnt/β-catenin target genes contain the sequence-specific Kaiso binding site in their promoters and Kaiso acts as a transcriptional repressor toward these genes, antagonizing β-catenin-mediated transcriptional activation [81, 82]. p120 interacts

with Kaiso's zinc finger domain to prevent Kaiso-DNA binding; thus p120 antagonizes Kaiso's transcriptional repression, facilitating Wnt/β-catenin signaling.

The signals that govern the p120-Kaiso interaction and their combined regulation of transcription are largely unclear. However, a recent study in Xenopus indicates that p120 is stabilized by Frodo, a downstream target of Wnt/Dsh signaling [82]. This stabilization resulted in the inhibition of Kaiso-mediated repression of Wnt/β-catenin target genes. Consistent with this, Wnt3a signaling controls the transcriptional activity of Kaiso, via the CK1ε-mediated phosphorylation of p120 [83]. Thus p120's transcriptional activity may be regulated by Wnt signaling: another point of signaling convergence between p120 and β-catenin. The signals that govern the cytoplasmic vs. nuclear localization of Kaiso are also not well characterized, although it is suggested that the cell's microenvironment plays a role. As with p120's other roles in mediating cadherin stabilization and Rho GTPase signaling, cell context undoubtedly affects the ability of p120 to regulate transcription; the details regarding how remain to be elucidated. Finally, an interaction of nuclear p120 with the transcriptional repressor Gli-similar 2 (Glis2) was also reported, although its functional significance is still unclear [84].

3.4 Regulation of cell motility by p120-Rho GTPase signaling

Low-level p120 overexpression via a retroviral delivery system promotes cell motility [12]. This effect is mediated by Rho GTPases, and possibly their subsequent effects on integrin-mediated signaling after adhesion to the extracellular matrix (ECM) [85–87]. The effects of p120 on cell migration depend on the specific cadherins expressed in the cells [88]. How mesenchymal cadherins promote cell migration is currently unclear, but it may involve the p120-mediated activation of Rac1 signaling and subsequent actin reorganization [88–90]. Inhibition of RhoA by cytoplasmic p120 in cells expressing mesenchymal cadherins may also promote migration by allowing the re-organization of the actin cytoskeleton [88, 91]. In contrast, in cells expressing E-cadherin, p120 is important for the pro-migratory signaling of either EGF or HGF, via a mechanism that involves p120's N-terminus and RhoA activation [92]. In the case of breast cancer cells expressing E-cadherin, p120-mediated Rac1 activation was required for HER2/ErbB2 induced cell migration [89]. Therefore, it seems that p120 and Rho GTPase signaling are involved in the motile behavior of cells expressing mesenchymal cadherins or activated receptor tyrosine kinases, but the specific mechanisms are not well defined.

3.5. p120 and microtubules

It has been shown that p120 interacts with the microtubule network in several ways, both directly and indirectly. This interaction supports a bi-directional functional role, by regulating a) p120 trafficking in the cell, and b) microtubule bundling and tethering to the junctions. p120 interacts with microtubules directly via the ARM repeats and this interaction is mutually exclusive to E-cadherin binding [13]. p120 can also bind to microtubules indirectly via it's N-terminus [13, 14, 16]. An interaction with conventional kinesin heavy chain controls p120 trafficking [13, 14, 93] and nuclear accumulation [13, 94]. Dynein-mediated tethering of microtubules to the junctions enables delivery of p120 to these sites [93].

Additionally, p120 promotes microtubule stabilization, bundling, and tethering to the junctions [13, 15, 95], via a novel binding partner called PLEKHA7 [16, 56]. This interaction is again mediated by the N-terminus of the p120 molecule. p120 and PLEKHA7 form a complex at the AJs that connects to the microtubule network through the microtubule minus end capping protein Nezha and the KIFC3 motor [16]. The p120-PLEKHA7-Nezha interaction links E-cadherin-p120 complexes with the minus ends of non-centrosomal microtubules, stabilizing AJs [16]. Importantly, prior studies have shown that non-centrosomal microtubule organization is essential for AJ symmetry and planar polarity in Drosophila [96], while vertically-aligned non-centrosomal polyglutamylated microtubules are required for polarized vesicle transport and the establishment of epithelial cell polarity [97]. Interestingly, the interaction of p120 with PLEKHA7 occurs selectively at mature AJs in polarized Caco2 cells, and not at perijunctional cadherin puncta [16]. Overall, p120-microtubule cross talk is essential in mediating both the intracellular localization of p120 and in stabilizing the microtubule-junctional architecture.

4. p120 in Cancer

Normal cells inhibit their growth and migration when they adhere to each other. These properties are progressively lost in tumor cells, contributing to increased rates of cell proliferation and migration. The processes imply that adhesion-triggered signaling events regulate both cell growth and motility. Based on its ability to regulate E-cadherin function, p120 is expected to act as a tumor suppressor, by stabilizing junctions. Surprisingly, recent studies show that signaling events downstream of p120 and cadherins are crucial for the anchorage-independent growth of tumor cells, as well as for Src-mediated transformation [19, 20]. Therefore, p120 exerts both pro-tumorigenic as well as anti-tumorigenic functions, an intriguing behavior (Figure 2). Despite many remaining questions that will provide the basis for future investigations, several important factors that have been so far identified to affect p120 behavior in cancer are discussed here.

4.1 p120 and cadherin switch in tumor growth and progression

During gastrulation, ingressing epithelial cells in the ectoderm undergo EMT, which allows them to adopt new cell fates, migrate through the basement membrane away from the epithelial monolayer, and establish new tissues. This normal developmental process overcomes the structural requirements for epithelial cell survival, and is characterized by a switch in the expression of epithelial versus mesenchymal markers, including cell-cell adhesion receptors of the cadherin family. During tumor progression though, differentiated epithelial cells undergo a similar transition, lose their characteristic phenotype and acquire mesenchymal properties, becoming motile and invasive. A major event that influences this transformation is an essential change in the type of junctions that these cells form and the subsequent signaling events that are triggered. This change is mainly due to a switch in the type of cadherins expressed. In particular, while epithelial cells primarily express E-cadherin, which is a well-established tumor-suppressor, expression of E-cadherin is decreased or diminished during EMT, whereas expression of other cadherins, such as N-cadherin or Cadherin-11 is induced. These cadherins are collectively described as "mesenchymal" cadherins. This switch results in membrane ruffling, immature junctions,

induced cell motility and invasiveness, all hallmarks of tumorigenic transformation. Indeed, it is well established in a variety of epithelial cancers, such as invasive lobular breast carcinoma and diffuse-type gastric adenocarcinoma, that loss of E-cadherin expression is correlated with increased infiltrative growth and tumor cell motility (reviewed in [98, 99]). Several signaling pathways and mediators of this process have been described so far. For example, E-cadherin engagement during cell-cell adhesion regulates the levels of the cyclin kinase inhibitor p27 [100, 101] and suppresses signaling from a variety of receptor tyrosine kinases (RTKs), including EGF receptor (EGFR) and c-Met [102], resulting in decreased rates of cell growth. In addition, cadherin-mediated cell-cell adhesion suppresses cell migration by regulating the activity of Rho family GTPases via p120 [88].

Since p120 stabilizes E-cadherin complexes, it is expected that it should exert anti-tumorigenic activities (Figure 2). In several cases of p120 deletion in vivo, the main effect was severe epithelial barrier malfunction and induction of inflammation, via RhoA and NFkB activation in the skin [103], or by accumulation of COX-2 expressing neutrophils in the colon [47, 104]. Solid evidence of the tumor-suppressor function of p120 in vivo was given in the salivary gland, where p120 deletion resulted in neoplastic lesions, as a result of severe adhesion defects and E-cadherin destabilization [48]. The tumor suppressor function of p120 was further established recently, where in vivo deletion of p120 resulted in invasive squamous neoplastic lesions in the oral cavity, esophagus, and forestomach [105]. p120 deletion was again accompanied by induction of pro-inflammatory events (Figure 2), such as activation of NFkB, Akt, Stat-3 and production of the granulocyte macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), monocyte chemotactic protein-1 (MCP-1), and of the tumor necrosis factor a (TNFa) from tumor-derived cells. These events were followed by desmoplasia and accumulation of immature myeloid cells, both hallmarks of the development of a tumorigenic microenvironment. The above findings demonstrate the anti-tumorigenic and anti-inflammatory properties of p120, in vivo, and are associated with E-cadherin expression in the cells.

In agreement with the animal data, several immunohistochemical studies argue that p120 expression is downregulated in certain tumors [106–110]. Also, p120 is transcriptionally downregulated by FOXC2 in non-small cell lung cancer (NSCLC) [111]. Downregulation of p120 would be expected to decrease E-cadherin-mediated adhesion, and possibly relieve inhibition of RTKs. The full ramifications of this condition are not yet understood, however, the combined loss of cell adhesiveness, increased RTK signaling, and altered Rho signaling may have profound effects on the aggressiveness of these tumors. Consistent with this, p120 knockdown in lung cancer cell lines enhanced migration and invasiveness [112]. The correlation of p120 loss with increased tumor aggressiveness [108] also suggests that in a background of other cancer-related mutations, p120 loss may promote metastasis.

On the other hand, p120 is also affected by cadherin switch during EMT and is an essential effector of the downstream signaling events induced by growth factor receptors, which lead to tumorigenesis (Figure 2). Early studies argued that p120 mislocalizes to the cytoplasm as a result of E-cadherin loss during tumor progression [113, 114]. For example, during EMT in colon cancer cells, E-cadherin is gradually lost and p120 gets mislocalized to the cytoplasm, where it inhibits Rho activity [91]. In this case, cytoplasmic p120 correlated

strongly with later-stage tumors, lymph node metastasis and reduced survival in a cohort of colorectal cancer cases [91]. Cytoplasmic p120 was also strongly correlated with late-stage pancreatic cancer [115]. Abnormal overexpression and cytoplasmic localization of p120 was associated with poor prognosis in lung squamous cell carcinoma and adenocarcinoma [109]. In ovarian cancer, gonadotropin-releasing hormone (GnRH) receptor expression promotes an E- to P-cadherin switch, p120 mislocalization, induction of Rac1 and Cdc42 activities, and tumor invasiveness [90]. A potential mechanism by which p120 can directly affect the cadherin switch was also proposed. Cells that were forced to overexpress R-cadherin had reduced levels of E- and P-cadherin. This phenomenon was the result of competition between the different cadherins for p120 binding. R-cadherin overexpression resulted in sequestration of p120 to R-cadherin, which led to E-cadherin de-stabilization, endocytosis and degradation [116].

p120 is not only affected by a cadherin switch and E-cadherin loss, but is also essential in influencing downstream pathways towards induction of cell motility and invasiveness [19, 88]. p120-dependent regulation of Rho GTPase signaling is one example (Figure 2). Upon loss of E-cadherin, cytoplasmic p120 was implicated in a murine model of infiltrating lobular carcinoma of the breast (ILC) via interaction and inhibition of Mrip, an antagonist of Rho/Rock function and ROCK activation [76]. Experiments in breast and kidney cancer cell lines have revealed that in addition to loss of E-cadherin, an accompanying increase in mesenchymal cadherin expression, and p120/mesenchymal cadherin-associated Rac1 signaling is required for the invasiveness of E-cadherin deficient tumor cells [88]. Therefore, cadherin switching during EMT and deregulated Rho GTPase signaling promote cell migration and tumor growth.

Surprisingly, in some cases p120 exerts tumorigenic activities even when E-cadherin is present. For example, ERBB2 overexpression in normal mammary epithelial MCF10A cells activates Rac1 and Cdc42 and induces cell migration and invasion in a p120-dependent manner, without any changes in E-cadherin expression [89]. In this study, p120 overexpression in an ERBB2-overxpressing but weakly metastatic breast cancer cell line, the BT474 cells, dramatically induced its ability to metastasize *in vivo* [89]. Rac1b, a constitutively active isoform of Rac1, required p120 binding in order to promote cell motility, in the E-cadherin positive mouse mammary epithelial cells [71]. In another recent example, p120 was shown to be essential in the progression of a highly lethal form of cancer, the inflammatory breast cancer (IBC) [117]. In IBC, overexpression of the translation initiation factor eIF4GI promotes p120 overexpression via specific IRES. As a result, E-cadherin is stabilized and favors IBC progression, likely by supporting the formation of the characteristic emboli of IBC. Therefore, in IBC, p120-E-cadherin complexes are overexpressed and pro-tumorigenic. Furthermore, aberrantly cytoplasmic p120 in invasive breast carcinomas that express P-cadherin, correlated with poor survival, especially when E-cadherin was also present [118]. In agreement, in cases of ILC where E-cadherin is still expressed, p120 is cytoplasmic, suggesting mis-regulation of the cadherin-p120 complex [119]. p120 is also required for the collective migration of A431 cells in a three-dimensional medium (matrigel or collagen) upon EGFR stimulation. This function of p120 is attributed to cadherin stabilization required for the cells to maintain their junctions in order to collectively migrate and invade [120].

Overall, although there is solid evidence that p120 exerts both pro-tumorigenic and anti-tumorigenic functions depending of the type of cadherin present, there are a number of cases where this relationship is not linear, indicating that the full mechanistic details of these functions remain to be elucidated.

4.2 p120 isoform-specific tumorigenic events

An extra layer of complexity in identifying the role of p120 in cancer progression involves the relative expression of p120 isoforms (Figure 2). We have reported that full-length p120 and N-terminally truncated p120 isoforms differentially affect Rho GTPase activities, cell migration and tumor cell invasion, while a p120 isoform switch during tumor progression predicts metastatic disease [69]. More specifically, the long isoform 1A is capable of promoting invasiveness, whereas isoform 4A cannot, and its expression was found to correlate with renal cancer micrometastasis. Both isoforms are able to bind RhoA downstream of HGF and Met signaling, however, only the cooperative binding of RhoA to a central binding domain and to the N-terminus that is contained in the long isoform 1A stabilizes RhoA binding and effectively inhibits its activity [69]. By efficiently suppressing the RhoA-ROCK pathway and by activating Rac1, isoform 1A induces migration and invasiveness of breast cancer cells. Furthermore, examination of p120 isoform expression across a series of breast cancer cell lines showed that the luminal, more invasive cells show high expression of the large isoforms 1 and 2, whereas isoform 3 is almost universally expressed [121]. Overexpression of isoform 3A attenuated proliferation of colon cancer HT-29 cells, in agreement with the aforementioned observations, although a role of this isoform in aberrant mitosis and induction of polyploidy was also proposed [122].

A new transcription factor named Zeppo 1 was shown to deregulate junction formation and promote lung metastases of breast cancer, by inducing the expression of the pro-tumorigenic isoform 1 of p120 [123]. Other studies also showed that overexpression and mis-localization of p120 isoform 1 strongly correlates with poor outcome in lung cancer [124].

Interestingly, both isoforms 1 and 3 are able to induce cell proliferation, via different pathways that ultimately affect Cyclin D1. In particular, p120 isoform 1A can induce Cyclin D1 via β-catenin increased expression and signaling, whereas isoform 3A does it by decreasing the levels of Kaiso in the nucleus, thereby relieving Cyclin D1 repression [125]. This study highlights the complexity in attributing pro-tumorigenic functions to particular p120 isoforms, indicating that these functions may be context-dependent, and clearly not yet fully delineated.

4.3 p120 phosphorylation correlates with cancer occurrence

p120 is phosphorylated by Src family kinases at a number of tyrosines within its N-terminus, such as Y96, Y112, Y228, Y257, Y280, Y291, Y296, and Y302 [33]. EGFR also phosphorylates p120 at Y228, without Src being the necessary intermediate [126] (Figure 2). A number of studies have showed that p120 phosphorylation correlates with tumorigenic events. In particular, phosphorylation of p120 at pY228 correlates positively with oral squamous cancer [127]. Phosphorylation of p120 in several tyrosine and serine sites is inversely related to cadherin activation and adhesion strengthening [55].

In addition to tyrosine, serine phosphorylation of p120, which is mainly induced by PKC [58], has been also associated with pro-tumorigenic events. Phosphorylation of p120 isoform 3 at S288 increased Kaiso binding and promoted lung cancer cell invasion [75]. Furthermore, Wnt signaling induced phosphorylation of p120 at S268 and S269, dissociating it from E-cadherin and subsequently promoting Kaiso sequestration and activation of downstream Wnt signaling events [83]. Overall, the evidence points towards a pro-tumorigenic role for p120 phosphorylation, however, the mechanistic details of this action are still unclear.

4.4 Regulation of anchorage independent growth by p120

A hallmark property of cells that are transformed with oncogenes is their capacity to grow under anchorage-independent conditions. The acquisition of anchorage independent growth (AIG) correlates strongly with tumorigenicity *in vivo,* and is thought to be a key transition in tumor progression associated with aggressive disease. In essence, AIG allows epithelial cells to grow in the wrong environmental context. Loss of dependence to the basement membrane for survival and progression through the cell cycle are properties associated with AIG that are closely related to the induction of tumor growth associated with carcinoma in situ, a key transition during human tumor progression.

Normal epithelial cells undergo apoptosis when suspended, in a process termed anoikis. There is evidence that suggest a link between cadherin-mediated adhesion and anoikis. In the context of the colonic epithelium, cadherin-mediated cell-cell junctions prevented anoikis via the activation of Src and PI3K-dependent signaling pathways [128, 129]. Two separate studies argue that 1) anoikis can be prevented by maintaining cell-cell junctions, who are often lost upon cell suspension, and 2) that anoikis is induced by the EGF-stimulated disruption of enterocytic cell-cell contacts upon detachment from the ECM [130]. However, cellular context and epigenetic modifications may play a critical role in the overall effect of E-cadherin depletion on anoikis. For example, in the context of p53 mutations, loss of E-cadherin in mammary epithelial cells leads to ILC, due in part to anoikis resistance [76]. Overall, it is likely that intercellular adhesion-related signaling events can substitute for focal adhesion signaling and drive the transformed growth of tumor cells. Following up on the original observation that p120 is a transformation-relevant Src substrate, recent work has established that p120 is an essential mediator of the Src and Rac1 induced AIG [20] (Figure 2). p120 mediated suppression of the Rho-ROCK pathway was necessary for AIG induction driven by the overexpression of either Rac1 or Src [20]. The observation that ROCK is recruited to E-cadherin complexes by binding directly p120 [75], further establishes the connection between p120, ROCK and AIG.

Signaling downstream from ECM and focal adhesions cooperates with growth factors to induce a sustained activation of ERK/MAP kinase, increased expression of Cyclin D1, and cell cycle progression [131]. Interestingly, p120 is essential in bypassing the requirement for ECM induction of the ERK/MAP kinase pathway, and for Cyclin D1 activation of tumor cells grown in soft agar. This activation occurred in the presence of cadherin 11 but not of E-cadherin and was mediated by Rac1 activation [19]. The data supported a model where in the presence of E-cadherin p120 promotes the stabilization of E-cadherin complexes and

their ability to suppress Ras and Rac1 signaling, thus blocking cell growth. However, upon E-cadherin loss during EMT or tumor progression, the negative regulation of Ras is relieved, endogenous p120 induces Rac1 activation, constitutive activation of the ERK MAP kinase signaling pathway, cell cycle progression, and anchorage-independent growth [19].

Therefore, it is important to note that anchorage independence in epithelial cells is a complicated process, requiring both suppression of apoptosis and progression through the cell cycle via signaling events triggered by cell-cell contact. Despite many remaining questions that will provide the basis for future investigations, the current data argue that intercellular adhesion and p120 can regulate the actin cytoskeleton and the activity of mitogenic pathways under conditions of anchorage independence.

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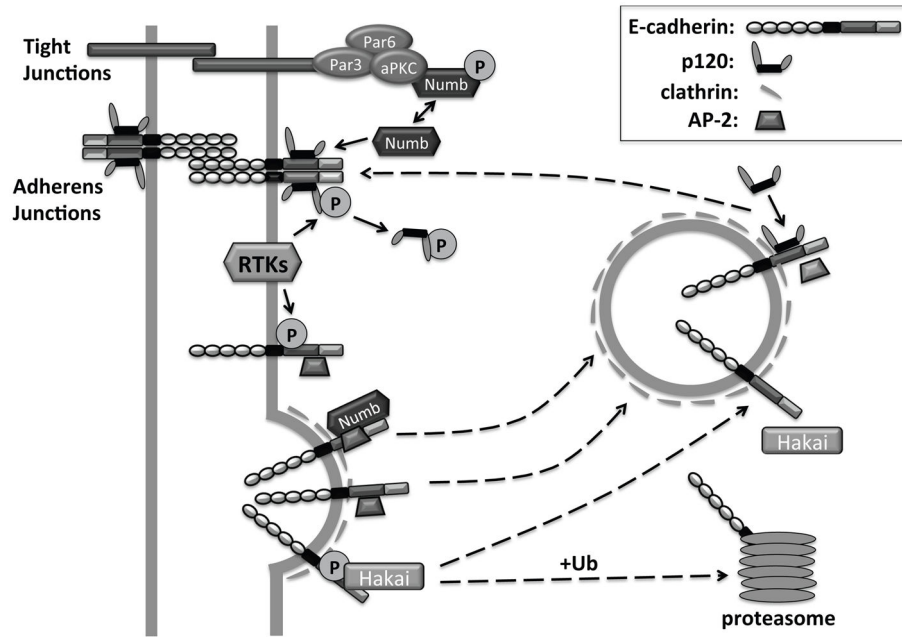
Figure 1. Cadherin stabilization by p120 catenin

p120 binding to the cadherin juxtamembrane domain stabilizes cadherins at the cell membrane and promotes the formation of adherens junctions. Removal of p120 from the cadherin complex can proceed via multiple mechanisms, including phosphorylation of p120 and/or E-cadherin downstream of receptor tyrosine kinases (RTKs), or association with non-phosphorylated Numb, a protein normally phosphorylated by aPKC. p120 dissociation uncovers an adaptor protein 2 (AP-2) binding motif, as well as a phosphorylation-dependent motif for the recruitment of the E3 ligase Hakai. AP-2 binding promotes clathrin-dependent endocytosis of E-cadherin. The endocytosed E-cadherin can be recycled back to the membrane, upon binding to p120. Alternatively, Hakai-induced ubiquitination of E-cadherin can lead to cadherin degradation in the proteasome.
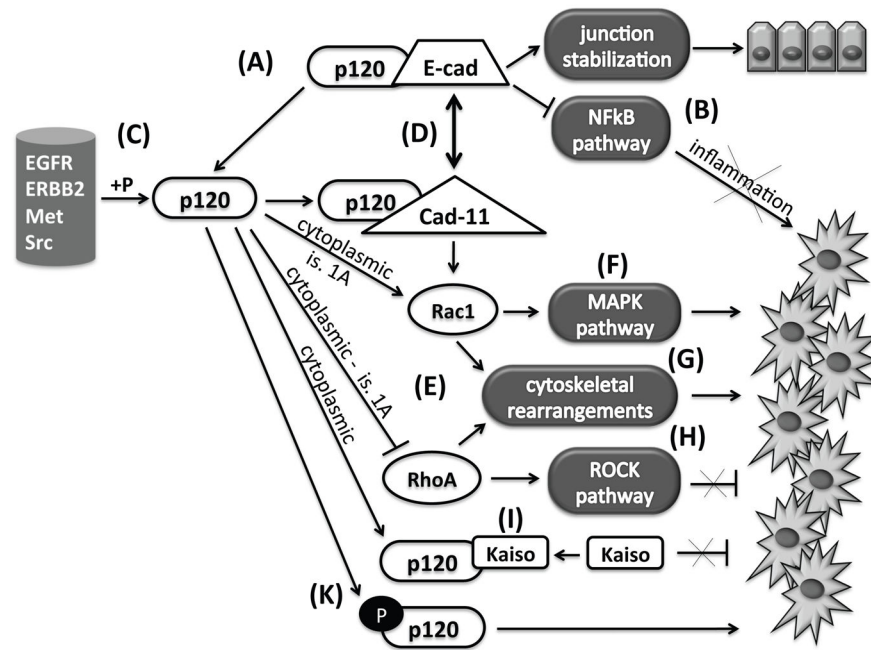
Figure 2. p120 catenin in cancer
Under normal conditions, p120 binds E-cadherin, stabilizes junctions (A), and suppresses inflammatory signaling pathways (B). However, upon phosphorylation by several kinases (C) or after cadherin switch (D), p120 induces pro-tumorigenic pathways, mainly by modulating Rho GTPase activities (E). These pro-tumorigenic events include activation of the MAPK pathway (F), cytoskeletal rearrangements (G) and suppression of the ROCK pathway (H). In addition, p120 cytoplasmic localization results in Kaiso sequestration to the cytoplasm and inactivation (I), also relieving transcriptional suppression of oncogenic signals. p120 phosphorylation is well correlated to tumorigenesis (K).