p27Kip1 in cell-cell adhesion and cell polarity
Theard, Delphine Francine

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2006

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 17-01-2019
Chapter 6

Summary, discussion and perspectives

Delphine Théard

Department of Cell Biology, section Membrane Cell Biology, University Medical Center Groningen, 9713 AV Groningen, The Netherlands
Summary

Discovered almost twenty years ago as a cyclin-dependent kinase inhibitor (CKI), p27 has been extensively studied since its loss is correlated with poor cancer prognosis. However, several studies have shown that p27 cytoplasmic localization contributes to a poor survival despite a high global expression. In addition, p27 has been implicated in processes like migration and apoptosis that do not require its activity as a CKI (Philipp-Staheli et al., 2001; Besson et al., 2004). In the studies presented in this thesis, we demonstrate for the first time the direct implication of p27 in various aspects of apical-basolateral cell polarity establishment.

In hepatocellular carcinoma HepG2 cells, the IL-6 family cytokine Oncostatin M (OSM) stimulates apical-basolateral cell polarity establishment in a protein kinase A-dependent manner by regulating membrane traffic to the developing apical surface (van der Wouden et al., 2002). In Chapter 2, we demonstrate that the effect of OSM on cell polarization relies on its ability to increase p27 expression levels thereby inhibiting cdk2 activity, thus causing an inhibition of G1-S transition. The latter is crucial for the effectiveness of OSM as modulator of polarity development: once the cells have entered S-phase, they become insensitive to the polarity-stimulating effect of OSM. Additionally, OSM-mediated recruitment of protein kinase A to the centrosomal region is abolished and the OSM-mediated activation of the protein kinase A-dependent transport route to the apical surface is prevented. This, however, can be rescued upon pharmacological inhibition of cdk2 activity. The ability of OSM to control p27-mediated cdk2 activity and, consequently, G1-S-phase progression is therefore crucial for this cytokine to regulate centrosome dynamics and membrane-directed traffic to the plasma membrane, and to stimulate cell polarity development.

OSM is a crucial cytokine for proper liver development. Particularly, in fetal rat hepatocytes, it was demonstrated that
OSM induces the formation of E-cadherin based Adherens Junctions (AJ; Matsui et al., 2002). In chapter 3, we demonstrate that OSM stimulates the phosphorylation of p27 on Serine 10, prompting investigations of the effect of Ser-10 mutants on cell-cell adhesion. Using different experimental approaches, we show that p27 phosphorylation on Ser-10 is required for Adherens Junction protein complex formation. Indeed, the overexpression of a non-phosphorylatable p27 mutant (S10A) prevents cell surface expression of the AJ proteins E-cadherin and β-catenin. By contrast, overexpression of wild-type or a phospho-mimetic p27 mutant (S10D) induces a hyper-adhesive phenotype. Additionally, we show that in cells expressing non-phosphorylatable p27, but not wild type or the phospho-mimetic mutant, p27 and β-catenin interact, while β-catenin does not interact with E-cadherin. Importantly, the opposing effects of the non-phosphorylatable and phospho-mimetic mutant do not appear to be related to p27 (mutant)-mediated cell cycle control. These data demonstrate for the first time that phosphorylation of the cell cycle regulatory protein p27 on Ser-10 directly controls the ability of epithelial cells to establish cell-cell adhesion.

For more than 10 years, E-cadherin was believed to be the cue initiating the biogenesis of the Adherens Junctions Complex, consisting of Adherens junctions (AJ) and Tight Junctions (TJ), between cells. AJ and TJ are presumed to assemble following initial cell-cell contact. In chapter 4, we show that in the p27S10A cell line (described in chapter 3), which does not express E-cadherin at the cell surface, apical-basolateral polarity establishment still occurs. These AJ-defective cells display every characteristics of an apical domain: TJ proteins are properly localized and form functional TJ. These structures provide a “fence”, which functions to segregate apical and basolateral proteins and lipids. They also provide a “gate” function to separate apical and basolateral extracellular milieus. Interestingly, the inability to form AJs precludes bile canalicular plasma membrane morphogenesis. Moreover, we observed a switch from
an indirect- to a direct-apical targeting pathway for the apical resident protein DPPIV, but not for 5’ nucleotidase, suggesting a regulatory role of cell surface-associated E-cadherin in the targeting of distinct apical proteins. How E-cadherin directs the different apical delivery pathways remains to be elucidated. Together, these data demonstrate that E-cadherin-mediated cell-cell adhesion is dispensable for apical lumen biogenesis per se but not for subsequent apical lumen remodeling, and may be involved in directing specific apical proteins and/or trafficking pathways to the basolateral domain.

In chapter 5, we have investigated the involvement of p27 and its Ser-10 phosphorylation site in apical plasma membrane organization at the (ultra)structural level. Overexpression of wild-type p27 or p27S10A results in an increase in the frequency and length of apical surface microvilli. By contrast, overexpression of p27S10D produces shortened and highly disorganized apical microvilli and typically electron-lucent apical lumens. The appearance of electron-lucent apical lumens is accompanied by a subapical accumulation of large, electron-dense late endosomes/multivesicular bodies (MVB) and secondary lysosomes. The effects of p27 Ser-10 mutants could not be attributed to its known effect on cell cycle control. Furthermore, inhibition of Rho signaling, a previously reported target of p27, does not affect the structural integrity of BC. However, inhibition of Rho signaling does cause the accumulation of electron-dense lysosomes, which are morphologically distinguishable from those seen in p27S10D-expressing cells. Together, our data implicate p27 and its Ser-10 phosphorylation site as a novel determinant in structural apical surface organization and late endosome/lysosome dynamics.

In conclusion, p27 plays a crucial role in the OSM-stimulated establishment of apical-basolateral polarity of HepG2 cells by regulating cdk2 activity and, in this way, clearly defines a careful programming of cell-cycle progression and polarity development. In addition, we demonstrate for the first time a
direct role for p27 and its Ser-10 phosphorylation status in cell-cell adhesion establishment, the structural organization of the apical surface, and MVB dynamics, which is not related to its well-defined role in controlling the cell cycle.

**Discussion and perspectives**

The literature concerning p27 is in constant evolution with the discovery of new interacting partners, implicating this protein in cellular processes not related to its CKI function. This thesis reveals the crucial role of p27 in hepatocyte polarization, not only by virtue of its inhibitory role on cdk2, but also by its ability to directly influence cell-cell adhesion, MVB dynamics and apical membrane integrity through Ser-10 phosphorylation. Nevertheless, numerous questions remain and extensive additional work is necessary to further clarify the essential functions and underlying mechanism(s) of this protein in these diverse cellular processes.

With regard to the cell cycle, OSM increases the cellular level of p27, which blocks cell cycle progression and sensitizes the cells to polarize. Also, p27 is phosphorylated on its Ser-10 in response to OSM, but this phosphorylation does not greatly influence the cell cycle, as evidenced by the (over)expression of different mutants, non-phosphorylatable or phospho-mimetic. How OSM governs p27 in adopting different functions, in either a cell cycle-dependent or independent manner requires further in depth investigations, also in terms of clarifying the specific role of OSM in polarity development. In particular, since phosphorylation appears as the most common mechanism by which the cell seems to regulate a distinct functional expression of p27, establishing the phosphorylation profile of p27 in response to OSM treatment could be a key experiment. In this context it is remarkable that the majority of studies concerning p27 phosphorylations usually focus on one or two phosphorylated
isoforms linked to only one particular cellular process. However, it seems reasonable to envision that different kinases can phosphorylate p27 on different sites and that it is this combination of phosphorylations which dictates the cell cycle-dependent or independent functions of the protein, in conjunction with a (likely phosphorylation-driven) distinct subcellular localization and/or stability.

The implication of Ser-10 phosphorylation in the regulation of cell-cell adhesion also deserves further investigation. As a first step, it will be of interest to determine which kinase is responsible for the phosphorylation of Ser-10. Experiments involving overexpression of hKis and Mirk/Dyrk1B, two kinases known to phosphorylate p27 on Ser-10, are currently in progress in our lab. Moreover, these two kinases act at different cell cycle stages, and it would thus be of interest to determine which molecular factors regulate their expression. In this context, it will be particularly challenging to determine whether compounds/factors leading to the enhancement of cell-cell adhesion and polarity, including OSM, could be involved in such a regulation.

p27 interacts directly with the small GTPase Rho, which by its action on the actin cytoskeleton promotes the formation of stress fibers and focal adhesions. Moreover, the p27 homolog in *Drosophila*, Dacapo, interacts with the GTPase Rap1, which interferes with cadherin-mediated cell junction formation and actin dynamics. In light of the clear link, demonstrated in the present study, between the overexpression of p27 Ser-10-to-Ala, β-catenin sequestration and the defect of E-cadherin-based AJ, it would thus be of major interest to investigate the potential relation between p27 and these two GTPases in relation to cell-cell adhesion and cell polarity development.

In addition to its role in cell-cell adhesion, β-catenin is a key player in the Wnt/Wingless signaling cascade, which is implicated in the epithelial-to-mesenchymal transition associated with cancer. In this thesis, it is suggested that p27 Ser-10
phosphorylation could act as a switch between the cell adhesion function of β-catenin and its signaling role in the Wnt pathway. Accordingly, it will be highly useful to determine the activity of these Wnt target genes in the different p27 (mutant) overexpressing cell lines.

AJ-deficient HepG2 cells display apical structures surrounded by functional tight junctions. This discovery is in contradiction with the prevalent dogma in cell-cell adhesion, stating that prior homotypic interaction between two E-cadherins of neighboring cells is the principle event in cell-cell adhesion establishment. Whether, the E-cadherin mislocalization in AJ-deficient cells is compensated by the expression of N-cadherin, as observed in other cancer cell lines, or whether the weaker interactions between two nectins suffice to tighten apposing membranes so that other close contacts are established, for example via desmosomes, remains to be determined. Yet this work, together with recent work from the laboratories of Peifer (on Drosophila) and Clevers (on unicellular polarity of intestinal cells), clearly revisits the concept of the requirement of AJ-dependent cell-cell adhesion in cell polarity development, and will evidently lead to new investigations on the chronology of events taking place during adhesion junctional complex establishment and its precise requirement and/or involvement in polarization.

Many questions remain unanswered concerning the role of Ser-10 phosphorylation in apical cell surface organization and endomembrane dynamics. However, the interaction of p27 with the microtubule-depolymerizing protein stathmin may be the key common link between these various processes. First, p27 interaction with stathmin results in the stabilization of the microtubule network, which has been reported to be involved in MVB dynamics and lysosomal secretion into the bile. Second, stathmin has a region within its sequence that is very similar to Lyst, a protein which, when mutated, causes defects in secretion from granules and lysosomes. Finally, stathmin has been reported
to interact with the tumor susceptibility gene Tsg101, a member of the ESCORT-I complex that promotes MVB formation by sorting ubiquitinated substrates into MVB. How p27 and its phosphorylation on Ser-10, stathmin, and the tsg101/ESCORT-I complex interact to regulate endomembrane dynamics and apical cell surface organization will need to be elucidated.

In conclusion, this study reveals new functions of p27, conferring novel and exciting facets to a protein that has been extensively studied in the context of cell cycle regulation in relation to cancer. The importance of p27 in the hepatocellular carcinoma cell line HepG2 is obvious from the work presented in this thesis, but the extension of these findings to other models will hopefully lead to a more general understanding of the global action of this protein in the intricate relationship between cell proliferation and cell polarization, two cellular processes intimately linked to cancer development.