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Close encounters of the RNAi kind: the silencing life of the adherens junctions

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Abstract

The adherens junction has been historically considered an essential structural component of epithelial tissues. Although primarily discussed as targets of signaling pathways responsible for cell fate and tissue remodeling, they have also emerged as critical signaling regulators in developmental processes or in disease progression. The recent discovery of a functional localized RNA interference (RNAi) machinery at epithelial adherens junctions revealed a new layer of signaling regulation that is directly associated with the structure itself. This and other findings also indicate that our view of the subcellular localization of RNAi requires revisiting. A number of questions emerge regarding the physiological role and the modes of regulation of the junctional RNAi machinery, pointing towards new directions of investigation.

Introduction

The adherens junctions are essential adhesive and architectural cell structures [1,2]. E-cadherin is the core adherens junction molecule in epithelial tissues and is associated through its cytoplasmic tail with numerous proteins that are collectively termed catenins [1,2]. The central role of E-cadherin in the formation and maintenance of the epithelial phenotype supported its function as a tumor-suppressor [3], which was further validated by firm experimental evidence. However, a series of intriguing findings revealed that E-cadherin can also promote survival and collective cell migration in tumors [4–8], suggesting a context-dependent tumor-promoting function. An effort to address this conundrum revealed the unexpected interaction of cadherin complexes with the core components the RNA interference (RNAi) machinery at the apical adherens junctions, also known as the zonula adherens [9[•],10[•],11]. Here, we summarize these novel findings, we put them in context and we discuss their significance and future directions.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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RNA complexes are a prevalent component of the adherens junctions

Recent work exposed an extensive interaction of cadherin complexes with RNA-binding proteins. In particular, all three essential complexes of the RNAi machinery have been identified to localize at the zonula adherens: a) the microprocessor and its main components DROSHA and DGCR8; b) the DICER-TRBP complex; and c) the core (Ago2, GW182, PABPC1) as well several accessory components of the RNA-induced silencing complex (RISC) [9^{••},10[•]] (Figure 1). These interactions are mediated through the p120 catenin binding partner PLEKHA7, which strictly localizes at the zonula adherens of epithelial cells and tissues [9^{••},10[•],11–13] (Figure 1). Repeated rounds of proteomics and subsequent pathway analyses in these studies confirmed RNA-binding proteins among the top functional groups interacting with PLEKHA7 [9^{••},10[•]]. Comprehensive E-cadherin proteomics have also shown that RNA-binding proteins comprise 25% of the overall cadherin proteome, further supporting the idea that this is an essential novel component of the adherens junctions that warrants to be fully explored [14[•]]. The core components of the RNAi machinery had been previously identified to localize in distinct subcellular compartments: the microprocessor in the nucleus and the DICER and RISC in the cytoplasm [15–22]. However, it is now evident that sub-populations of all three main RNAi complexes, as well as several of their miRNA and mRNA substrates, co-exist at the same subcellular compartment, the zonula adherens (Figure 1) [9^{••},10[•]]. Notably, certain miRNAs, such as members of the miR-30 and let-7 families or miR-24, seem to be both regulated by the junctional microprocessor and also associate with the junctional RISC [9^{••},10[•]]. A common characteristic of these miRNAs is that they are considered guardians of the epithelial phenotype and suppress promoters of cellular transformation, such as SNAIL1 and MYC [9^{••}, 10[•]]. Epithelial monolayers act as key physical and functional barriers that are indispensable for overall organismal health and function. Therefore, the ability of epithelial adherens junctions to regulate the processing and silencing activity of these guardian miRNAs may be providing epithelial cells with a rapid means to regulate protein expression, in order to maintain monolayer homeostasis. Still, several mechanistic questions remain unanswered, including the processing activity of the junctional DICER and the direct cross-talk between all the three junctional core RNAi complexes at the adherens junctions. Nonetheless, these studies demonstrated: a) that the localization of the RNAi machinery is specific to the apical zonula adherens and not observed at lateral areas of cell-cell contact; and b) that the junctional localization of RNAi complexes is required for the processing and function of certain miRNAs. For example, cytoplasmic re-localization of PLEKHA7 resulted in loss of the junctional RNAi machinery, similarly to PLEKHA7 depletion, whereas dismantling of the apical junctions by E-cadherin or p120 catenin depletion also replicated these effects [9^{••},10[•]]. Therefore, it seems that the apical zonula adherens possesses a unique role in the processing and function of a certain set of miRNAs that support epithelial cell homeostasis.

The subcellular landscape of RNAi is changing

The discovery of a functional microprocessor complex at the adherens junctions is in contrast to the previously prevailing notion that the microprocessor is solely a nuclear complex [15–19]. Similarly, RISC was previously widely considered a cytoplasmic complex [20–23] (Figure 1). It is likely that the junctional localization of the microprocessor and

RISC was not previously observed because of the cell models used in these studies, which were either fibroblasts or transformed cancer cell lines. These cell types are widely used and suitable for biochemical studies, however, they do not form mature adherens junctions. Indeed, the association of the microprocessor and RISC with the adherens junctions was revealed in well-differentiated cell lines, such as MDCK and Caco2 [9^{••},10[•]]. This localization was restricted at the zonula adherens, a specialized structure that typically appears at apical areas of cell-cell contact in polarized epithelial cells[1,2,9^{••},10[•]]. Nevertheless, the RNA expression, sequencing and protein fractionation data show that only subpopulations of the RNAi complexes and specific sets of miRNAs and mRNAs localize at the adherens junctions [9^{••},10[•]]. The majority of the microprocessor and of RISC seem to still localize in the nucleus and the cytoplasm, respectively [9^{••},10[•]]. Therefore, the localization and function of RNAi complexes to the junctions does not necessarily challenge the current dogma of miRNA biogenesis, but adds to it. Several questions stem from these observations and remain to be addressed, such as: a) the extend of the junctional localization of RNAi in differentiated epithelial cells and tissues; b) the percentage of miRNAs and mRNAs that are actually regulated by this mechanism in different cell types; c) the specific miRNAs and mRNAs that are regulated in each tissue, since it is expected that these will differ between different cell types; d) the extend that this mechanism influences cellular behavior; and e) the mechanisms that regulate junctional RNAi localization and function.

The discovery of a functional microprocessor at the adherens junctions was the first demonstration of the non-nuclear presence of this complex, under normal physiological conditions. Interestingly, it has been previously shown that viral infection can trigger a dramatic re-localization of the microprocessor to the cytoplasm, which is then recruited to process viral pri-miRNAs [24]. However, two more recent studies revealed the existence of alternatively spliced variants of DROSHA that possess full processing activity but localize in the cytoplasm [25^{••},26^{••}]. These studies also identified differential expression of DROSHA isoforms in different cell types, without this influencing the efficiency of miRNA processing [25^{••},26^{••}]. Collectively, the above indicate that subcellular localization of the microprocessor can indeed be non-nuclear and that this may be more prevalent than previously thought. It would be interesting to examine whether specific DROSHA isoforms localize to the adherens junctions. These findings also imply the existence of a currently unidentified mechanism of microprocessor and/or primary miRNA (pri-miRNA) transportation from the nucleus to the cytoplasm. It was recently reported that knockout of exportin 5 (XPO5), which is thought to mediate processed miRNA export from the nucleus, only modestly affects mature miRNA levels [27[•]]. This further supports the possible existence of either an alternative mechanism of nuclear miRNA export, or of a non-nuclear DROSHA-mediated pri-miRNA processing step. Another important question is how the microprocessor and RISC are eventually transported to the adherens junctions. Multiple studies have shown that a frequent avenue of intracellular RNA trafficking is the microtubule network [28–30]. However, nocodazole-mediated dismantling of the microtubules did not affect the junctional localization of the microprocessor or the RISC protein components[9,10]. Still, the effects of nocodazole treatment on miRNA and mRNA transport had not been assessed in these studies and this remains to be addressed. In addition, several studies have shown that RNAs and RNA-binding proteins are being transported through the

actin cytoskeleton [31–33]. Since PLEKHA7 depletion has a negative impact on the integrity of the cortical actin ring [9**], it would be important to examine the possibility that the actin cytoskeleton mediates localization of the RNAi complexes to the junctions.

Cellular RNAs are mostly local

The spatial subcellular distribution of miRNAs, mRNAs and RNA-binding complexes is not a novel concept. It has been primarily observed in neuronal cells, where localized mRNA storage and translation in dendrites and axons, as well as miRNA maturation, is now considered critical for processes such as synaptic activity or axon branching [34–36]. Numerous other examples also indicate that spatial distribution of RNAs in cells is the rule and not the exception. It has been shown that about 70% of mRNAs exhibit specific localization patterns in *Drosophila* embryos [37]. Similar work in other model systems, such as in ascidia, yeast, *Xenopus*, as well as in mammalian cells, confirmed that RNA localization is a widespread and conserved phenomenon [38]. Specifically regarding miRNAs, it is now well-documented that they co-exist with mRNAs, with components of the RNAi machinery and numerous other RNA-binding proteins in p-bodies or stress granules in the cytoplasm [39]. Specific sets of mRNAs are also found in granules in cell protrusions [40]. It has been postulated that p-bodies are sites where mRNAs are stored and remain silenced by miRNAs and RISC, so that they are readily available for translation upon corresponding stimuli [41**]. The co-existence of miRNAs, mRNAs, RISC and of numerous RNA-binding proteins at the adherens junctions [10*] is reminiscent of the composition of p-bodies and could similarly be a deposit of mRNAs that can be rapidly translated in response to changes in the status of the adherens junctions. However, this needs to be examined and could be an important step to determine the functional significance of this mechanism. Interestingly, two other encounters of RNA complexes with cell-cell adhesion components have been previously reported: a) plakophilin (PKP1), a p120 catenin family member that participates in desmosome formation, interacts with the translation factor EIF4A1 in the cytoplasm to promote its activity [42]; and b) localization of the β -actin mRNA at areas of cell-cell contact through the RNA-binding protein IGF2BP1 (also known as Zipcode Binding Protein 1, ZBP1) is required for adherens junction assembly [43]. The recent PLEKHA7-related findings revealed a much broader network of interactions between the adherens junctions and RNA complexes that hasn't been previously reported. Cumulatively, these findings support the notion that spatial subcellular RNA distribution is common, it is tightly regulated and is critical for cellular function.

What is the role of the junctional RNAi? How is it regulated?

Disruption of the RNAi localization to the junctions promotes pro-tumorigenic cell transformation[9]. In addition, PLEKHA7 is downregulated or lacks junctional localization in human patient samples from breast and kidney tumors [9**]. The data are consistent with the hypothesis that PLEKHA7 mediates the tumor suppressing function of apical E-cadherin complexes by tethering the RNAi components to the junctions. However, to what extent disruption of this mechanism could lead to tumor initiation or progression requires further examination. Currently missing are also the underlying causes of PLEKHA7 and junctional RNAi dysregulation in tumors. For example, it is not known how PLEKHA7 levels are

regulated and whether this occurs through a transcriptional or a post-transcriptional mechanism. It is also unclear why PLEKHA7 is mis-localized in many of these tumors [9^{••}]. Src activity opposes localization of PLEKHA7 and of the RNAi proteins to the junctions [9^{••},10[•]], which is consistent with previous findings showing that Src disrupts strong cell-cell adhesion [44] (Figure 2). Src is an oncogene frequently activated in many types of cancer downstream of receptor tyrosine kinases, therefore, it may be responsible for the dysregulation of the junctional RNAi machinery. In agreement, Src-dependent tyrosine phosphorylation of p120 is also frequent in tumors [45] (Figure 2). In these cases, it would be of interest to examine the signaling that leads to Src activation, such as EGFR activity. Intriguingly, a cross-talk between EGFR and Ago2 has been reported, but not at the junctions [46]. In particular, EGFR phosphorylates Ago2 at Y393 under hypoxic conditions, which results in inhibition of DICER-mediated miRNA loading to RISC and attenuation of miRNA biogenesis [46[•]]. In a similar study, it has been demonstrated that oncogenic KRAS binds and suppresses Ago2 silencing activity in the endoplasmic reticulum, to potentiate cancer cell aggressiveness [47[•]]. These studies were performed in fibrosarcoma or lung cancer cells, therefore, it would be interesting to examine if a similar EGFR-Ago2 and KRAS-Ago2 cross-talk exists in non-transformed epithelial cells and whether this disrupts the junctional localization and activity of RISC (Figure 2).

An intriguing question is the actual physiological role of the RNAi localization to the junctions of well-differentiated polarized epithelial cells. One possibility is that it serves as a “sensor”, through which cells identify the formation of mature adherens junctions during tissue remodeling or wound repair. This would be critical to rapidly suppress expression of proteins that stimulate cellular growth, junction remodeling, and de-differentiation, such as MYC, SNAIL1, and SOX2, in order to promote epithelial monolayer integrity and homeostasis. It could also serve as a localized mechanism of contact inhibition, in addition to other signaling pathways involved, such as YAP/TAZ signaling and the Hippo pathway [48]. Interestingly, a cross-talk of Hippo and the microprocessor was revealed to occur in the nucleus of keratinocytes [49]. In particular, it has been shown that YAP interacts with DDX17 (p72), an accessory component of the microprocessor, to suppress miRNA processing [49]. Increase in cell confluency promotes YAP re-localization to the cytoplasm, which results in relief of DROSHA suppression and increased miRNA processing and expression [49]. Since both YAP and other members of the Hippo pathway interact with junctional and polarity components [50,51], it would be important to examine their possible effects on the function of the junctional microprocessor (Figure 2). Both the junctional RNAi and the nuclear Hippo-microprocessor findings are in agreement with a report showing that increase in cell confluency promotes miRNA biogenesis [52]. Overall, given the known roles of the Hippo pathway and YAP/TAZ signaling as mechanosensors, it would be interesting to examine the contribution of mechanical stress on the regulation of the miRNA machinery at the adherens junctions. Taken together, these mechanisms underscore the role of miRNAs in epithelial cell behavior and in response to formation of mature cell-cell contacts. In addition to epithelial biology and given the similarities between epithelial and endothelial tissue organization, it would be important to investigate the potential presence and involvement of junctional RNAi in normal endothelial cell function and vascular disease.

Concluding remarks

In summary, the discovery of a localized RNAi mechanism at the adherens junctions seems to be an additional level of regulation of cellular homeostasis. This should be taken into account when studying mechanisms of cell fate and disease, in addition to high-throughput tools that examine gene expression or mutation status, which cannot assess this extra layer of subcellular complexity. The advantage that this mechanism confers to the cell may be that it enables rapid responses in miRNA biogenesis and mRNA translation, during junction remodeling. By affecting a select subset of miRNAs and mRNAs, this mechanism has the potential to globally affect cell behavior during development, wound healing, or cancer. Further investigation of this localized RNAi function will provide a better understanding of its roles in normal cell behavior and human disease, as well as its potential as a therapeutic target.

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Figure 1. The adherens junctions recruit and regulate RNAi complexes

The E-cadherin - p120 catenin (p120) partner PLEKHA7 recruits the microprocessor complex (DROSHA, DGCR8), the DICER complex and the RISC (Ago2, GW182, PABPC1), as well as miRNAs and mRNAs at the apical zonula adherens (ZA) of epithelial cells. Through this mechanism, PLEKHA7 regulates processing and levels of a set of miRNAs to suppress expression of mRNAs that promote cellular transformation, thus maintaining the normal epithelial phenotype.

Figure 2. Potential modes of regulation of the junctional RNAi machinery

It is likely that oncogenes disrupt the function of the junctional RNAi. First, it has been shown that active Src opposes localization of the microprocessor and RISC components to the junctions and promotes expression of pro-tumorigenic markers through p120 phosphorylation at the basolateral cadherin complex. It has been also shown that activated EGFR and KRAS can bind and suppress Ago2 and RISC activity in the cytoplasm; it would be interesting to examine whether this Ago2 suppression by EGFR and KRAS also disrupts the function of the junctional RISC during tumor progression. In addition, YAP can suppress the processing activity of the microprocessor in the nucleus; since there is extensive cross-talk of YAP and the Hippo pathway with the junctions, it would be valid to interrogate their involvement in the regulation of the junctional microprocessor.