Ion channels/transporters as epigenetic regulators? —a microRNA perspective

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MicroRNA (miRNA) alterations in response to changes in an extracellular microenvironment have been observed and considered as one of the major mechanisms for epigenetic modifications of the cell. While enormous efforts have been made in the understanding of the role of miRNAs in regulating cellular responses to the microenvironment, the mechanistic insight into how extracellular signals can be transduced into miRNA alterations in cells is still lacking. Interestingly, recent studies have shown that ion channels/transporters, which are known to conduct or transport ions across the cell membrane, also exhibit changes in levels of expression and activities in response to changes of extracellular microenvironment. More importantly, alterations in expression and function of ion channels/transporters have been shown to result in changes in miRNAs that are known to change in response to alteration of the microenvironment. In this review, we aim to summarize the recent data demonstrating the ability of ion channels/transporters to transduce extracellular signals into miRNA changes and propose a potential link between cells and their microenvironment through ion channels/transporters. At the same time, we hope to provide new insights into epigenetic regulatory mechanisms underlying a number of physiological and pathological processes, including embryo development and cancer metastasis.

ion channel, miRNAs, epigenetic, microenvironment

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Cells in their native tissues reside in an extracellular milieu, the soluble microenvironment composed of or influenced by an assembly of various factors, such as trophic or growth factors, neurotransmitters, inorganic ions, pH, oxygen, osmolarity and temperature [1,2]. Altogether, these factors converge via a multitude of intracellular signaling pathways that ultimately govern whether a cell divides, differentiates, or dies. Moreover, the complexity of cellular responses to environmental stimuli develops in a temporal and spatial

manner spanning over many orders of magnitude [3,4]. Thus, it is not surprising that accumulating evidence has shown that environmental cue is of paramount importance for the normal function of cells, disruption of which may lead to aberrant cellular phenotypes and development of diseases [5].

MicroRNAs (miRNAs) are noncoding RNAs with pleiotropic effects dependent on posttranscriptional regulation of gene expression [6]. By interfering with multiple transcripts, miRNAs have the potential to regulate virtually all

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cellular mechanisms, and have been identified as key players in producing rapid adaptation to changing environmental conditions [7,8]. Interestingly, several recent studies have established a link between cellular microenvironment and specific miRNAs [9–11], indicating that miRNAs may be a key to understand not only the adaptive responses of the cells to their microenvironment, but also the mechanisms underlying disease processes induced by adverse stimuli, such as hypoxia in cancer. However, one remaining open question is how signals from the microenvironment surrounding the cells are transduced into miRNA alterations. To this end, while few studies have provided mechanistic insights, recent findings from our group and others have given rise to the notion that ion channels/transporters may function as miRNA regulators by sensing environmental changes and triggering downstream signaling pathways. In this review, we aim to provide an overview of alteration of miRNA and ion channels/transporter expression profiles in response to physiological and pathological microenvironmental changes. Particularly, recent evidence supporting an important role of ion channels/transporters in transducing extracellular signals into miRNA alterations is analyzed to provide new insights into epigenetic regulatory mechanisms underlying a number of physiological and pathological processes.

1 MiRNAs and alterations of their expression in response to microenvironmental changes

MiRNAs are short single-stranded RNAs of approximately 20–24 nucleotides in length, which regulate the expression of target genes at the post-transcriptional level by binding to their 3'-untranslated regions [6]. Since miRNAs do not require perfect complementarity to their targets, each miRNA can potentially target thousands of different mRNAs [12,13]. Consequently, while a relatively small amount of miRNAs (over 1000) have been identified in the human (<http://www.mirbase.org>), they are able to regulate about as much as 20%–30% of the human genome and involved in virtually all biological processes, including cell growth, differentiation, proliferation, apoptosis and metabolism [14]. Moreover, miRNAs also play key roles in modifying chromatin structure and participating in the maintenance of genome stability [15]. Therefore, compared with other mechanisms involved in gene expression, miRNAs are directly involved in the fine-tuning or quantitative regulation of gene expression in a more dynamic and versatile manner. Recently, specific miRNAs have been reported to be associated with the pathogenesis and development of various diseases, such as diabetes, cancer, cardiovascular diseases, neurodegenerative diseases and developmental disorders [16]. As a result, miRNAs are emerging as a new type of molecular markers for the diagnosis and therapeutics of various diseases.

MiRNA-based posttranscriptional regulation is extremely sensitive to the stimuli in microenvironment, such as low oxygen (hypoxia), variations in pH, osmolarity, temperature and ion concentrations. For example, the ability to sense and respond to hypoxia is of fundamental importance to aerobic organisms, whereas dysregulated oxygen homeostasis is a hallmark in the pathophysiology of cancer, neurological dysfunction, myocardial infarction, and lung disease [17]. Since 2006, a multitude of reports have demonstrated that specific miRNAs are involved in hypoxic response and contribute to the repression of biologically important genes by low oxygen tension from a variety of different organisms, cell types, and disease states [18–21]. While these studies have described more than 90 hypoxia-regulated miRNAs, of note, miR-210 is the only miRNA consistently upregulated in all published studies, in both normal and transformed hypoxic cells [9,20–23]. Consistently, at least in breast cancer, miR-210 levels are found to be correlated with a genetic signature of hypoxia, suggesting that overexpression of this miRNA in tumor is the consequence of decreased oxygen tension in the microenvironment [24,25].

In the case of electrically excitable cells or tissues, intracellular events triggered by ion dynamics can affect the single-cell or even cell cluster behavior in terms of contractile properties and/or gene expression [26]. For example, the work on miRNAs has been focused especially on hypertrophy, heart failure, and the control of electrical activity in the heart. Several studies have reported that some miRNAs are either increased (e.g., miR-195) [27] or decreased (e.g., miR-1, miR-133) [28] in response to dysregulated ion dynamics during pathological heart conditions. Importantly, alterations in the expression of miRNA may underlie the process producing the dysfunctional cardiac excitability and arrhythmic death [29]. Another intriguing finding of miRNA regulation by the microenvironment comes from the recent reports showing that miRNAs participate in cellular responses to osmotic stress in mammalian cells. Huang *et al.* [30] reported that miR-200b and miR-717 were highly tonicity-sensitive and able to regulate the stability of osmotic response element binding protein (OREBP) mRNA and protein *in vitro* and *in vivo*. Another group has also illustrated that the expression of multiple miRNAs was significantly altered following the exposure of human articular chondrocytes to hyperosmotic conditions [31]. Apart from these, a study investigating the possible role of miRNAs in the regulation of mammalian hibernation found that miR-1 and miR-21 were both significantly increased in the kidneys of hibernating versus euthermic animals, providing the first evidence of differential expression of miRNAs in response to temperature control in mammals [32]. Taken together, these studies support the notion that miRNA regulation in response to microenvironmental changes may contribute to cellular physiology and abnormality of such regulation may be involved in pathological processes.

2 Ion channels/transporters and their cross-talk with the microenvironment

In mammals, ion channels/transporters are expressed in almost all cell types including epithelial cells, endothelial cells, neurons and even immune cells. The steady-state maintenance of highly asymmetric concentrations of the major inorganic cations and anions is a major function of ion channels and active transporters, which regulate the movement and distribution of ions across the lipid barrier in the plasma membrane [33]. Localized at the apical or basolateral membrane of the cells, ion channels/transporters are in a strategic position to sense and transmit extracellular signals into the intracellular machinery. Often, this transmission is mediated by their direct or indirect interaction with ECM proteins, such as integrins [34] and laminin [35]. Apart from that, ion channels are readily responsive to various environment stimuli, such as pH and hypoxia. For example, a potent control of intra- and extracellular pH can be considered as one of the cancer hallmarks [36]. Recent work from S. Reshkin's group [36,37] showed that extracellular environment is mainly acidified by the Na^+/H^+ exchanger NHE1 and the $\text{H}^+/\text{lactate}$ cotransporter that are typically active in cancer cells. What is more, NHE1 also regulates the formation of cell structures that mediate tumor cell migration and invasion. Another molecule that links extracellular acidification to tumor growth and invasiveness is the $\text{Na}^+/\text{HCO}_3^-$ cotransporter, which can directly bind to carbonic anhydrase (CA)-IX and IV [38,39] that are known to be hypoxia-inducible proteins that can control tumor pH by maintaining a steep outward CO_2 gradient, with alkaline intracellular and acidic extracellular pH [40,41]. More interestingly, recent study on colon cancer cells has demonstrated that hypoxia inductive factor α (HIF-1 α) suppresses the expression of the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-activated anion channel conducting both Cl^- and HCO_3^- , by direct repressive binding to its promoter [42]. These observations suggest possible cross-talk between ion channels/transporters and their microenvironment, such as low oxygen tension and acidic pH in cancer [33].

In the context of reproductive tract, an optimal fluid microenvironment is considered to be crucial for successful reproductive events occurring along the genital tract, such as sperm capacitation, fertilization, embryo development and implantation [43,44]. For instance, during blastocyst implantation, the volume and composition of uterine fluid have been reported to undergo significant changes to facilitate blastocyst attachment and embedding [45]. Disturbance of the fluid microenvironment, i.e., by bacterial infection or in disease condition such as cystic fibrosis (CF), results in implantation failure and infertility [44,46–48]. Fluid absorption and secretion across the reproductive tract epithelia largely depends on electrolyte transport through ion

channels such as epithelial sodium channel (ENaC) and CFTR [49–52]. Furthermore, CFTR is also known to be involved in bicarbonate transport either directly [53] or indirectly [54], which is important for activation of cAMP-dependent signaling pathway [53,55] in addition to pH regulation. In the last decade, various studies from our laboratory have demonstrated that normal function of CFTR and/or ENaC is critical for a variety of reproductive events such as sperm capacitation [53,56] and implantation [57,58], defects or dysregulation of which lead to a number of pathological conditions, such as ovarian hyperstimulation syndromes, hydrosalpinx and infertility [44,59]. These observations suggest that ion channels/transporters may respond to microenvironmental changes and play an important role in various physiological and pathological processes.

3 Potential involvement of ion channels/transporters in the regulation of miRNAs

The findings that both ion channels and miRNAs are extremely sensitive to the cellular microenvironment suggest the possibility of the two working together to bring about cellular changes in response to environmental changes. Indeed, several lines of evidence have indicated that ion channels/transporters may function as mediators linking signals from extracellular microenvironment to intracellular miRNA alteration which subsequently triggers a cascade of intracellular signaling that influences physiological or pathological processes. For example, our recent study has demonstrated that CFTR is involved in mediating the effect of extracellular HCO_3^- on regulation of miR-125b expression during preimplantation embryo development [60]. While it was known for a long time that HCO_3^- is required for embryo development, the exact mechanism remained elusive. Using a mouse model, we demonstrated that the effect of HCO_3^- on preimplantation embryo development could be suppressed by CFTR inhibitor or gene knockout. Furthermore, removal of extracellular HCO_3^- or inhibition of CFTR reduced miR-125b expression in 2 cell-stage mouse embryos. These results have revealed a critical role of CFTR in signal transduction linking the environmental HCO_3^- to activation of miR-125b, which targets p53 and p21, during preimplantation embryo development (Figure 1). In another recent study, we demonstrated that during embryo implantation, a protease known to be released from implanting embryos activated ENaC, which triggered Ca^{2+} influx leading to upregulation of COX-2 and PGE_2 release required for implantation [61]. Interestingly, we also found that the expression of two COX-2-targeting microRNAs, miR-199a and miR-101 were also altered during implantation (unpublished data). In addition, blocking or knocking down uterine ENaC in mice resulted in implantation failure

Figure 1 Working model for the regulation of early embryo development by CFTR/ HCO_3^- -dependent activation of miR-125b. CFTR mediates the influx of HCO_3^- ion directly and/or indirectly by cooperating with an anion exchanger. The influx of HCO_3^- activates sAC, an enzyme that converts ATP to cAMP, which in turn activates PKA, triggering the nuclear shuttle of NF κ B, a transcription factor known to regulate the expression of miR-125b. Induction of miR-125b expression by CFTR-mediated HCO_3^- influx maintains the dormancy of p53, which is required for early embryo development (originally published in *Cell Research* [60], with permission to reproduce from *Cell Research*).

accompanied with altered levels of uterine COX-2, miR-199a and miR-101. These results have indicated a previously undefined role of ENaC and possible involvement of miRNAs in regulating PGE_2 production and release required for embryo implantation, the defect of which may lead to miscarriage. Taken together, these results have indicated the importance of ion channels in the regulation of miRNAs in normal physiological conditions, dysfunction of which may lead to clinical manifestations.

Interestingly, by comparing wild type and mutant (DF508)-CFTR lung epithelial cell lines, Bhattacharyya *et al.* [62] recently found that 22 miRNAs were differentially expressed in CFTR defective cells. Among them, the expression of miR-155 was more than 5-fold elevated in CF IB3-1 lung epithelial cells compared with control IB3-1/S9 cells, which was suggested to be responsible for the enhanced expression of IL-8 and exaggerated inflammatory response observed in cystic fibrosis (CF). The association of CFTR and miR-155 was further validated by the clinical study showing that miR-155 was highly expressed in lung epithelial cells and circulating neutrophils from CF patients, indicating possible involvement of miR-155 in the development of CF lung disease. While it has been well established that mutations of CFTR result in a plethora of CF symptoms including lung inflammation, pancreas insuffi-

ciency, intestinal obstruction and infertility [63], the pathogenesis of various CF diseases remains obscure. Since the degree of disturbance in pH or ion concentration caused by CFTR defect may vary depending on individuals and circumstances, which could result in distinctive changes in epigenetic profile involving different sets of miRNAs even though CF patients may share a common mutation of CFTR. In addition, a single miRNA may target multiple genes, including those so-called modifier genes implicated in CF pathogenesis. Thus, defects in CFTR may lead to a multitude of clinical manifestations and varied disease severity through miRNA alterations. This may explain the complexity of CF disease processes and provide a rationale for varied degrees of severity observed in CF.

Another interesting finding from these studies is that some of the miRNAs are regulated by both CFTR and hypoxia (Table 1), such as miR-155, miR-21 and miR-23, all of which are implicated in cancer development [64,65]. Since HIF- α can directly bind to CFTR promoter and transcriptionally repress CFTR expression [42], it is plausible that CFTR mediates the effect of hypoxia on cancer progression through alteration of miRNAs. In other words, the hypoxia-induced downregulation CFTR may be responsible for the miRNAs alteration seen in various cancers. Indeed, our recent study investigating the role of CFTR in the de-

velopment of prostate cancer has revealed a direct association of CFTR with miR-193b expression in prostate cancer [95]. MiR-193b has been recently identified as an epigenetically regulated putative tumor suppressor in various types of cancer [66,67] and shown to target urokinase-type plasminogen activator (uPA) [68]. In our study, while CFTR knockdown was shown to promote the malignant phenotype of prostate cancer cells, overexpression of miR-193b significantly reversed the CFTR knockdown-enhanced proliferation, migration, or invasion and completely abrogated the CFTR knockdown-elevated uPA activity, as expected of uPA being a target of miR-193b. These results have clearly revealed a previously undefined tumor suppressing role of CFTR and its involvement in regulation of miR-193b in prostate cancer development. In breast cancer, miR-155 has been implicated in the TGF β -induced epithelial-mesenchymal transition process and cancer metastasis [69]. Given that CFTR can be downregulated by TGF β [70], it might be possible that CFTR mediates the effect of TGF β on EMT and cancer metastasis through its action on miR-155 and thus the downstream EMT-associated target genes in breast cancer. Since miRNAs play critical roles in the development of various cancers and CFTR can regulate a wide variety of miRNAs including those known to be involved in cancer development (Table 1), it is possible that the anti-tumor effect of CFTR is mediated through its action on miRNAs.

How do ion channels induce epigenetic changes? Take CFTR for example, as an anion channel, CFTR affects Cl⁻ and HCO₃⁻ concentrations in and out of the cells which may lead to activation of miRNA-mediated signaling pathways. Interestingly, CFTR-mediated HCO₃⁻ transport has been shown to activate a soluble form of adenylyl cyclase and

cAMP-dependent pathway in sperm [53] and lung epithelial cells [55], which may lead to activation of some cAMP dependent transcription factors, such as CREB, and thus transcription of related miRNAs. As discussed earlier, our recent study has shown that miRNA expression is greatly influenced by extracellular HCO₃⁻ concentration [60], and that CFTR manipulation affects miRNA expression profile in prostate cancer cell lines [95]. Besides, defective or dysregulated CFTR can activate NF- κ B, which has been demonstrated to be one of the major transcriptional factors to activate miRNAs [71,72]. Interestingly, we have also found that CFTR knockdown leads to enhanced activation of NF κ B in cancer cell lines [95]. In addition, we have shown that NF κ B inhibitor reverses CFTR knockdown-enhanced cancer progression [95]. Moreover, we have also shown that activation of miR-125b during preimplantation embryo development was mediated by sAC/PKA-dependent nuclear shuttling of NF- κ B (Figure 1) [60]. Taken together, the evidence provided suggests that ion channels may transcriptionally regulate miRNA expression through activation of transcriptional factors in response to the changes in extracellular environment.

Finally, it is also worth noting that CFTR has been demonstrated to be repressed by 12 miRNAs in the Caco-2 cell line [73]. Among them, miR-145 and miR-494 regulate CFTR expression by directly targeting discrete sites in the CFTR 3-UTR [73]. Thus, it appears that CFTR expression may also be dynamically regulated by miRNAs, as a feed-back mechanism involved in the regulatory circuit originated from microenvironment signals, which may in turn activates intracellular signaling pathways to maintain cellular homeostasis, details of which requires further investigation.

Table 1 Summary of microRNAs related to hypoxia and ion channels/transporters

MicroRNA	Symbol	Related to hypoxia	Related to ion channels or transporters
miR-155	MIRN155	Babar <i>et al.</i> 2011 [76]; Bruning <i>et al.</i> 2011 [77]	CFTR: Bhattacharyya <i>et al.</i> 2011 [62]
miR-21	MIRN21	Han <i>et al.</i> 2012 [78]; Yang <i>et al.</i> 2012 [79]; Redova <i>et al.</i> 2011 [80]	CFTR: Bhattacharyya <i>et al.</i> 2011 [62] L-type Ca ²⁺ channel: Carrillo <i>et al.</i> 2011 [81] TRPV1: Thilo <i>et al.</i> 2010 [82]
miR-23b	MIRN23B	Liu <i>et al.</i> 2010 [83]	CFTR: Bhattacharyya <i>et al.</i> 2011 [62]
miR-27	MIRN27	Thulasigam <i>et al.</i> 2011 [84]	CFTR: Bhattacharyya <i>et al.</i> 2011 [62]
miR-494	MIRN494	Ghosh <i>et al.</i> 2010 [85]	CFTR: Gillen <i>et al.</i> 2011 [73]; Megiorni <i>et al.</i> 2011 [86] SLC12A2: Gillen <i>et al.</i> 2011 [73]
miR-103	MIRN103	Kulshreshtha <i>et al.</i> 2007 [9]	Cav1.2- LTC: Favereaux <i>et al.</i> 2011 [87]
miR-26a	MIRN26A	Kulshreshtha <i>et al.</i> 2007 [9]	L-VGCC: Shi <i>et al.</i> 2009 [88]
miR-328	MIRN328	Guo <i>et al.</i> 2012 [89]	L-type Ca ²⁺ channel: Lu <i>et al.</i> 2010 [90]; Guo <i>et al.</i> 2012 [89] CFTR: Bhattacharyya <i>et al.</i> 2011 [62]
miR-31	MIRN31	Peng <i>et al.</i> 2012 [91]; Liu <i>et al.</i> 2010 [92]	CFTR: Bhattacharyya <i>et al.</i> 2011 [62]
miR-192	MIRN192	Kulshreshtha <i>et al.</i> 2007 [9]	CFTR: Bhattacharyya <i>et al.</i> 2011 [62]
miR-30a	MIRN30A	Guimbellot <i>et al.</i> 2009 [19]	L-type Ca ²⁺ channel: Rhee <i>et al.</i> 2009 [93]
miR-101	MIRN101	Cao <i>et al.</i> 2010 [94]	CFTR: Megiorni <i>et al.</i> 2011 [86]
miR-193b	MIRN193B	Guimbellot <i>et al.</i> 2009 [19]	CFTR: Xie <i>et al.</i> 2012 [95]
miR-125b	MIRN125B	Kulshreshtha <i>et al.</i> 2007 [9]	CFTR: Lu <i>et al.</i> 2012 [60]

4 Conclusion and perspective

Although recent breakthroughs have been made in the understanding of the role of miRNAs in epigenetic regulation of cellular response to cellular microenvironment, the mechanistic insight into how extracellular signals can be transduced into alteration of miRNAs is still lacking to a large extent. Accumulating evidence indicates that ion channels/transporters are emerging as key players in the cross-talk between cells and their surrounding microenvironment. We propose that ion channels/transporters located at the cell surface may function as epigenetic regulators by sensing environmental changes and transducing the micro-environmental signals into miRNA alteration, which subsequently leads to cellular adaptive responses in health and disease. To this end, ion channels have been reported to be associated with other epigenetic mechanisms apart from miRNAs, such as DNA methylation [74] and histone modification [75], reinforcing the importance of these molecules in the regulation of a wide variety of biological processes. Considering the fact that epigenetic research is paving the way for many new breakthroughs in the prevention, diagnosis and treatment of human diseases, focused efforts on the detailed mechanisms linking the environment and epigenetic responses in physiological/pathological conditions have an immense potential in improving human health and welfare. The potentials of ion channels/transporters can be further enhanced by the fact that they are highly accessible cell surface molecules and hence represent ideal drug targets. The investigation of the effect of ion channels on miRNA expression and the underlying signaling pathways may open up new revenue of therapeutic targets for the treatment of ion-channel related diseases, such as CF. We can expect that once the disease-specific miRNAs are identified, the validation of novel targets within a disease pathway of interest may lead to novel therapeutic strategies.

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