Ion Channels in Bone Morphogenetic Protein Signaling

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Abstract

How a single fertilized egg develops into a complex multicellular organism is one of the great mysteries of life. Developmental biology textbooks describe cascades of ligands, receptors, kinases, and transcription factors that designate proliferation, migration, and ultimately fate of cells organized into a multicellular organism. Recently, it has become apparent that ion channels are integral to the process of developmental signaling. Ion channels provide bioelectric signals that must intersect with the known developmental signaling pathways. We review some evidence that bioelectric signaling contributes to bone morphogenetic protein signaling.

Keywords: channelopathies, bone morphogenetic protein, BMP, inwardly rectifying potassium channels, ion channels and pumps, bioelectric signaling

Introduction

ONE OF LIFE'S GREAT MYSTERIES is how cells communicate to orchestrate movement, division, and cell fate choices to generate a complex multicellular organism. An elegant genetic screen in *Drosophila melanogaster* revealed signaling cascades that include ligands, receptors, kinases, and transcription factors that are essential for segmentation and patterning of an embryo.¹ Upon these foundational discoveries, developmental biologists have assembled conserved networks of interactions that are essential for patterning a multicellular organism. Textbooks and thousands of research articles detail the undeniable contributions of components of canonical developmental signaling pathways.

More recently it has become apparent that bioelectric signals work together with these canonical developmental signals for cell–cell communication to correctly specify morphogenesis. Bioelectric signals are created by variations in ion channel and ion pump activity and establish the cellular transmembrane potential (V_{mem}). Foundational studies from Michael Levin's laboratory have shown that the bioelectric pattern of cells within a developing tissue is important for the pattern and size of the developing tissue.^{2–5} Changing this bioelectric pattern can result in drastic changes in morphological development as profound as the formation of ectopic eyes in *Xenopus laevis*.⁶ Changes in the transmembrane potential pattern also affect mammalian craniofacial development, suggesting that bioelectric signals play a conserved role in morphological development.⁷

Development is conserved. Mutations that disrupt KCNJ2, encoding the inwardly rectifying potassium channel Kir2.1, cause morphological abnormalities such as cleft palate, micrognathia (small jaw), hypertelorism, low set ears, and digital defects such as syndactyly, clinodactyly, and brachydactyly.^{8,9} Mutations that disrupt the Cav1.2 (CACNA1C) calcium channel are associated with similar craniofacial and digital abnormalities in Timothy's syndrome.¹⁰ Mutations that disrupt another inwardly rectifying potassium channel called GIRK2 (KCNJ6) are associated with abnormal craniofacial development, including high arched palate, microcephaly, and lipodysorphy.¹¹ Mutations that disrupt a voltage-gated potassium channel Kv10.1 (Eag, KCNH1) are associated with stereotypical facial features, clinodactyly, and hypoplasia or aplasia of fingernails and toenails.^{12,13} Mutations that disrupt a two-pore potassium channel called TASK3 (KCNK9) are associated with a high-arched or cleft palate, short mandible, high eyebrows, a narrow forehead, a short philtrum, and cleft soft palate as part of Birk-Barel syndrome.^{14,15} Gain of function and loss of function mutations in the TRPV4 channel are associated with skeletal dysplasias.^{16–19} Although not all of these mutations that disrupt ion channel function have been shown to be causative, the fact that disruption of homologous ion channels in more than one organism is associated with morphological abnormalities suggests that these mutations contribute to the morphological defects.

Ion Channels in Human Development

Morphological features associated with mutations found in human patients suggest that the role for ion channels in de-

Ion Channels in *Drosophila* Development

A screen in *D. melanogaster* has revealed that a wide variety of ion channels are involved in developmental patterning.²⁰ Of 177 ion channels screened in *Drosophila*, 44

significantly contributed to wing development.²⁰ The high number of channels identified to contribute to morphogenesis in this screen suggests that ion channels likely play a broad role in patterning. Interestingly, ion channels of many different types including calcium, sodium, potassium, chloride, and ligand-gated ion channels were among those identified in the screen, revealing that the ion channels that impinge upon developmental pathways are not limited to a single category.²⁰

Many of the wing phenotypes observed upon disruption of the 44 ion channels identified in this screen were mild, possibly because ion channels often belong to families that are able to compensate for each other when a single member is disrupted. When one channel is deleted or knocked down, other channels can become upregulated in response, masking the phenotypes of the channel disruption.^{21,22} Indeed, within the 44 identified, we found that ion channels belonging to smaller families had more severe phenotypes than those belonging to larger families that could potentially be compensating for each other.²⁰ The ability of ion channels to compensate for each other means that the number of ion channels involved in development may be even higher than this screen revealed, as it is possible that compensation may mask the effects of single channel disruptions.

The Ion Channel Kir2.1 and Bone Morphogenetic Protein Signaling

Understanding how the bioelectric signals intersect with established signaling pathways is a key question. Some clues can be taken from a mouse lacking the inwardly rectifying potassium channel, Kir2.1, encoded by the gene *Kcnj2*. Several lines of evidence suggest that Kir2.1 function may specifically impact bone morphogenetic protein (BMP) signaling. The BMPs signaling pathway is among the essential conserved developmental cues that are used repeatedly throughout morphological development. Work in *Drosophila* lends insight into the mechanism by which Irk channels may impact BMP signaling. BMPs such as *Drosophila* Dpp are members of the transforming growth factor (TGF)- β superfamily and follow a stereotypical signaling cascade.²³ BMP ligands are released from the BMP-producing cells. BMP ligands bind to a complex of type 1 and type 2 serine threonine kinase receptors.²⁴ Upon ligand binding, the type 2 receptor activates the type 1 receptor.²⁵ The activated type 1 receptor phosphorylates receptor-associated Smads (1/5/8), which interact with Smad 4 and translocate into the nucleus to affect target gene expression.^{26–28}

Results consistent with a link between Kir2.1 and BMP family members are compelling. First, the Kir2.1 knockout mouse *Kcnj2*^{KO/KO} has phenotypes that are consistent with the channel's involvement in BMP signaling. For example, similar to mice in which BMP signaling components have been removed from the cranial neural crest, *Kcnj2*^{KO/KO} mice have hypoplastic maxilla, hypoplastic mandibles lacking the coronoid process, a cleft palate, and hypoplastic frontal bones leaving an enlarged fontanelle.^{29–31} Loss of Kir2.1 leads to similar limb patterning defects as loss of BMP ligands from the limb buds.^{32,33}

Second, Kir2.1 and BMP signaling are both required in the cranial neural crest cells for the proliferation of the mesenchyme of the palatal shelves.^{29,34,35} Third, E13.5 *Kcnj2*^{KO/KO} craniofacial tissues show a reduction in read-outs of BMP

signaling such as phosphorylation of Smads1/5/8 and expression of BMP transcriptional targets.²⁹ However, TGF- β signaling seems to be intact in *Kcnj2*^{KO/KO} tissue.²⁹ Furthermore, inhibition of Kir2.1 orthologs (Irk channels) in *Drosophila* also leads to phenotypes reminiscent of loss of a *Drosophila* BMP called Dpp.^{21,25,36–38} Inhibition or loss of Irk channels also reduces *Drosophila* Smad phosphorylation and expression of a Dpp transcriptional target.²¹ Together these lines of evidence suggest that Kir/Irk channels are important for efficient BMP signaling.

Mechanism of Kir2.1 Effect on BMP Signaling

Irk channels are required in the Dpp-producing cells of the *Drosophila* wing disk, but not required in the Dpp-responding cells for efficient Dpp signaling.³³ Inhibition of Irk channels in the Dpp-producing cells does not reduce the amount of Dpp produced.³³ However, without the channel's function, the Dpp is not released in a regulated pulsatile manner, suggesting that Irk channels regulate the release of Dpp.³³ In neurons, Irk channels regulate the transmembrane potential, which, in turn, affects intracellular calcium levels that can drive neurotransmitter release.³⁹ Inhibition of Irk channels reduces the amplitude and duration of native increases in intracellular calcium in Dpp-producing cells in the *Drosophila* wing disk, suggesting that Dpp release may be regulated by ion channel function and intracellular calcium levels.³³ BMP acts noncell autonomously. The ligand cannot perform its function if it is not released properly. If similar mechanisms regulate BMP and neurotransmitter release, a role for multiple types of ion channels in morphogenesis becomes apparent. It remains to be determined whether this model applies to other ion channels.

It is clear that ion channels contribute to morphological development of structures that we do not consider to be composed of excitable cells. The mechanism by which bioelectric signals intersect with canonical developmental signaling pathways is an area with exciting open questions.

Author Disclosure Statement

No competing financial interests exist.

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