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Review

Epigenetic regulation of gap junctional intercellular communication: More than a way to keep cells quiet?

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ABSTRACT

The establishment of gap junctional intercellular communication is a prerequisite for appropriate control of tissue homeostasis. Gap junctions consist of connexin proteins, whereby a myriad of factors govern the connexin life cycle. At the transcriptional level, most attention has yet been paid to the classical *cis*/*trans* machinery (i.e. the interaction between transcription factors and regulatory elements in connexin gene promoter regions) as a gatekeeper of connexin expression. In the last few years, it has become clear that epigenetic processes are also essentially involved in connexin gene transcription. Major determinants of the epigenome include histone modifications and DNA methylation, and recently, microRNA species have also been described as key regulators of the epigenetic machinery. In the present paper, the emerging roles of epigenetic events in the control of connexin expression, and consequently of gap junctional intercellular communication, are reviewed. Besides an updated theoretical background concerning gap junctions and epigenetic phenomena, we provide an in-depth overview of their interrelationship and we demonstrate the clinical relevance of the topic.

Contents

Abbreviations: 4-Me$_2$N-BAVAH, 5-(4-dimethylaminobenzoyl)aminovaleric acid hydroxamide; 4-PB, 4-phenylbutyrate; AP1, activator protein 1; ATP, adenosine trisphosphate; BRMS1, breast cancer metastasis suppressor 1; cAMP, cyclic adenosine monophosphate; CBP, CREB-binding protein; CL, cytoplasmic loop; CpG, cytosine–guanine; CT, cytoplasmic carboxy tail; Cx, connexin; DNMT, DNA methyltransferase; DNMTi, DNA methyltransferase inhibitor(s); EL1–2, extracellular loop 1–2; ERK1/2, extracellular signal-regulated kinase 1/2; GJIC, gap junctional intercellular communication; HAT, histone acetyltransferase; HDAC(s), histone deacetylase(s); HDACi, histone deacetylase inhibitor(s); HMBA, hexamethylene bisacetamide; IP$_3$, inositol trisphosphate; MAPK, mitogen-activated protein kinase; MBP, methylated DNA-binding protein(s); MeCP2, methyl-CpG-binding protein 2; miRNA(s), microRNA(s); mRNA(s), messenger RNA(s); NaB, sodium butyrate; NAD$^+$, nicotinamide adenine dinucleotide; ncRNA(s), non-coding RNA(s); NT, cytoplasmic amino tail; PKA, protein kinase A; PKC, protein kinase C; pre-miRNA(s), precursor microRNA(s); pri-miRNA, primary microRNA; REST, RE-1 silencing transcription factor; RISC, RNA-induced silencing complex; SAHA, suberoylanilide hydroxamic acid; siRNA, small interfering RNA; Sp1, specificity protein 1; TM1–4, membrane-spanning domain 1–4; TSA, Trichostatin A; UTR(s), untranslated region(s)

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1. Introduction

The maintenance of tissue homeostasis relies on the well-orchestrated interplay between extracellular, intracellular and intercellular communication networks. Direct communication between adjacent cells mainly occurs through vast arrays of intercellular channels, called "gap junctions", which are formed by members of the connexin family [1–4]. Numerous factors are known to drive physiological gap junction production and activity. Connexin gene transcription is ruled by specific sets of transcription factors [5]. Structural chromatin modifications are also known to alter transcriptional activity. Such epigenetic mechanisms have gained particular attention in a clinical context. Indeed, a major hallmark of cancer includes the drastically altered gene expression patterns, whereby oncogenes and tumor suppressor genes are typically induced and inactivated, respectively. Epigenetic processes play pivotal roles in the silencing of tumor suppressor genes [6,7]. The anticipated anti-tumor properties of connexin genes suggest that they may also be subject to such regulation [8,9]. In the current paper, we provide a state of the art picture of the current knowledge concerning the involvement of epigenetic mechanisms in connexin expression and function.

2. Gap junctions: key players in homeostatic control

2.1. Structure

Gap junctions, formerly known as *nexus* or *maculae communicantes*, are organized in plaques at the cell membrane surface and arise from the interaction of 2 connexons (hemichannels) from neighboring cells. Connexons, in turn, are built up by 6 connexin (Cx) proteins (Fig. 1). More than 20 mammalian connexin paralogues have been characterized, and they are named after their molecular weight [2–4,10,11]. They are expressed in a cell-specific way, with Cx43 being the most abundant connexin species [12]. Connexins share a common structure, consisting of 1 cytoplasmic carboxy tail (CT), 4 membrane-spanning domains (TM), 2 extracellular loops (EL), 1 cytoplasmic loop (CL) and 1 cytoplasmic amino tail (NT) (Fig. 1) [2–4,10,11]. Variety between connexins is mainly due to structural differences within the cytoplasmic areas [13]. Diversity also exists at the level of connexons, which can be composed of either 6 identical connexin subunits ("homomeric" connexons) or more than 1 connexin species ("heteromeric" connexons). This "connexin code" becomes even more complicated when considering gap junction architecture, as these channels consist of 2 heteromeric connexons ("heteromeric" gap junctions), 2 similar homomeric connexons ("homotypic" gap junctions), or 2 different homomeric connexons ("heterotypic" gap junctions) [14–16].

2.2. Function

Gap junctions provide a pathway for communication between adjacent cells, about 180 Å in length and 15 Å in diameter [17]. The flux of molecules through gap junctions, called "gap junctional intercellular communication" (GJIC), includes the passive flux of small and hydrophilic substances, such as cyclic adenosine monophosphate (cAMP), inositol trisphosphate (IP_3) and ions (Ca^{2+}) [18]. Although this seems a rather general route for exchange of essential metabolites between cells, GJIC is quite specific and the biophysical properties of a given gap junction type are determined by the nature of the composing connexin species (i.e. the connexin code) [14,18]. By doing so, GJIC is considered as a key mechanism in the control of tissue homeostasis. Numerous studies have actually demonstrated determinant roles of gap junctions in the occurrence of cell proliferation, cellular differentiation and apoptotic cell death [2,4,9,19–21]. Recent findings also point to GJIC-independent functions of connexins and connexons in these processes. Indeed, connexins as such can trigger gene expression, whereas gap junction hemichannels provide a pathway for the extracellular release of essential homeostasis regulators, like adenosine trisphosphate (ATP) [2,11,22–24]. Not surprisingly, connexins and their channels are frequently impaired upon disruption of the homeostatic balance. During cancer, for instance, physiological connexin expression patterns are abrogated, which typically burgeons into the drastic loss of GJIC [8–10].

2.3. Regulation

A plethora of regulatory mechanisms govern the connexin life cycle and GJIC. Short-term GJIC control, so-called "gating", is mediated by a number of factors, including transmembrane voltage, and Ca^{2+} ions and H^+ ions [14]. Among all gating mechanisms, phosphorylation has been most extensively studied. With the exception of Cx26, all connexins are phosphoproteins. Regulation of GJIC by connexin phosphorylation is quite complex, as the outcome of this posttranslational modification is both connexin-inherent and kinase-specific [25,26]. This is particularly true for Cx43, which can be phosphorylated by protein kinase A (PKA), protein kinase C (PKC) and members of the mitogen-activated protein kinase (MAPK) family, to name a few [26,27]. Long-term control of GJIC mainly concerns regulation at the transcriptional level of connexin expression. The structure of connexin genes is rather simple, namely a first exon containing the 5′-untranslated region (UTR), which is separated by an intron of varying length from a second exon, bearing the complete coding sequence, and the 3′-UTR [5,12,28]. Yet, 2 major exceptions to this common gene structure have been reported. First, the coding region can be interrupted by introns, such as in the case of Cx36 and Cx57. Second, different 5′-UTRs can be spliced in a consecutive and/or alternate manner (e.g. Cx32) [5,12,28,29]. Connexin gene promoters contain several binding sites for both ubiquitous transcription factors, like activator protein 1 (AP1) and specificity protein 1 (Sp1), and tissue-specific transcription factors [5]. In addition, epigenetic mechanisms are also essentially involved in connexin gene transcription, as will be outlined in the following sections.

3. Epigenetics: key mechanisms in transcriptional control

3.1. Introduction

A central dogma in molecular biology is that gene expression is governed by transcription factors which interact with specific DNA sequences, a process known as *cis*/*trans* regulation. In the last decades, however, it has become more than clear that other processes are also essentially involved in transcriptional control. These mechanisms are commonly referred to as epigenetic events and can be defined as mitotically and meiotically heritable changes in gene expression that are not coded in the DNA sequence itself [30]. Thus far, 2 major epigenetic regulatory mechanisms have been characterized, namely histone modifications and DNA methylation. Recently, microRNA (miRNA) species have been described as crucial regulators of epigenetic events [30–39]. For this reason, miRNAs are considered