Available online at www.sciencedirect.com

SCIENCE DIRECT

Biochimica et Biophysica Acta 1711 (2005) 208–214

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

http://www.elsevier.com/locate/bba

Review

Gap junction- and hemichannel-independent actions of connexins

Jean X. Jiang*, Sumin Gu

Department of Biochemistry, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA

Received 19 April 2004; received in revised form 6 October 2004; accepted 7 October 2004
Available online 22 October 2004

Abstract

Connexins have been known to be the protein building blocks of gap junctions and mediate cell–cell communication. In contrast to the conventional dogma, recent evidence suggests that in addition to forming gap junction channels, connexins possess gap junction-independent functions. One important gap junction-independent function for connexins is to serve as the major functional component for hemichannels, the un-apposed halves of gap junctions. Hemichannels, as independent functional units, play roles that are different from that of gap junctions in the cell. The other functions of connexins appear to be gap junction- and hemichannel-independent. Published studies implicate the latter functions of connexins in cell growth, differentiation, tumorigenicity, injury, and apoptosis, although the mechanistic aspects of these actions remain largely unknown. In this review, gap junction- and hemichannel-independent functions of connexins are summarized, and the molecular mechanisms underlying these connexin functions are speculated and discussed.

Keywords: Gap junction- and hemichannel-independent; Connexin; Cell proliferation; Tumorigenicity; Cell differentiation

Contents

1. Introduction

Gap junctions comprised of connexins form transmembrane channels connecting cytoplasms of adjacent cells, which allow ions, small metabolites, and second messengers to translocate from cell to cell (for review, see Refs. [1,2]). This type of intercellular communication is known to be essential for various physiological and pathophysiological functions, such as cell growth, proliferation and differentiation, tissue homeostasis, tumorigenicity, wound healing, etc. Up to now, approximately 20 types of connexin molecules have been reported in the mouse and human [3]. Connexin mutations have been identified to be related to several diseases, such as X-linked Charcot–Marie–Tooth disease, congenital deafness and skin disorders, and congenital cataracts caused by mutations in Cx32, Cx26, Cx46 and 50, respectively. More than 160 mutations of Cx32 have been found [4], some of which lead to complete loss of functional

* Corresponding author. Tel.: +1 210 567 3796; fax: +1 210 567 6595.
E-mail address: jiangj@uthscsa.edu (J.X. Jiang).

0005-2736/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2004.10.001

channels, while others form functional channels with certain abnormalities in channel behavior. Recently, several reports revealed other unconventional functions of connexins. In addition to being a component of gap junctions, connexin molecules can form hemichannels, which are un-apposed halves of the gap junction channels. The actions of connexins that form hemichannels have been discussed in detail elsewhere (for review, see Ref. [5]). In this review, we focus on the roles of connexins that are gap junction- and hemichannel-independent.

A study indicating that connexins may have separate, non-gap junction-related functions was reported in 1995 [6]. Since then, the molecular mechanism of this novel, significant type of action by connexins has started being investigated and elucidated. Increasing evidence suggests gap junction-independent function of connexins in cell growth and proliferation, tumorigenicity, differentiation, injury, and apoptosis.

2. Gap junction-independent functions of connexins on cell growth and tumorigenicity

It has long been known that gap junction intercellular communication (GJIC) contributes to the maintenance of normal cell growth and that the rate of cell growth is inversely correlated with the extent of GJIC [7,8]. Disruption of communication often results in abnormal cell growth and tumors. Therefore, the role of gap junctions and connexins is suggested in tumor suppression [9–11]. Additionally, aberrant connexins are most common in tumor tissues, such as the reduction in connexin expression and/or aberrant localization of connexin. Despite some of the arguments as to whether GJIC is involved in the control of cell growth, there is unequivocal evidence to show that connexins can suppress cell growth both in vitro and in vivo.

There is increasing evidence for gap junction-independent roles of connexins in the control of cell growth and the suppression of tumorigenicity (for summary, see Table 1). Mesnil et al. [6] observed that among cells transfected with various connexin genes, there was no correlation between their GJIC and tumorigenicity. GJIC levels were significantly higher in tumors induced by injecting cells transfected with Cx26 in nude mice although all of the connexin genes (Cx43, Cx40, and Cx26) examined could establish GJIC in HeLa cells. The report by Huang et al. [12] shows that transfection of the Cx43 gene into human glioblastoma cells reversed the transformed phenotype of these tumor cells; however, the tumor suppression by Cx43 was unrelated to the activity of GJIC determined by Lucifer Yellow dye coupling. Moreover, tumor-suppressive effect of connexins is more connexin species-specific than their activity in cell coupling [12,13]. Two reports from Yamasaki's group further show that certain mutants of Cx26 and Cx43 exert dominant negative effects on cell growth and tumorigenicity, but such effects are independent of the actions on GJIC assessed by dye transfer of Lucifer Yellow [14,15]. In one study, three mutated Cx26 genes (C60F, P87L and R143W) were expressed in HeLa cells that contained the wild-type Cx26 gene, and were GJIC-competent and non-tumorigenic. Interestingly, mutants, Cx26-P87L and Cx26-R143W, enhanced the tumorigenicity of the HeLa Cx26 cells in nude mice without any changes in GJIC. On the other hand, expression of the Cx26-C60F mutant reduced the GJIC level without affecting their growth in vivo [14]. In another study, two Cx43 mutants (L160M and A253V) were used to examine dominant-negative effects of Cx43 mutants on cell growth control exerted by wild-type Cx43 in C6 cells [15]. The mutant Cx43-L160M diminished in vitro growth-suppressive functions and GJIC capacity of wild-type Cx43 while it showed only weak dominant-negative effects on in vivo tumor-suppressive function. In contrast, the mutant Cx43-A253V presented a strong dominant-negative effect on both in vitro cell growth and in vivo tumor suppression exerted by wild-type Cx43 but not on GJIC. In the communication-proficient rat bladder carcinoma BC31 cell line, dominant negative mutants derived from the third transmembrane domain, Cx43-K162A, Cx43-E166E, and Cx43-T154Y, all blocked cell coupling; but only Cx43-T154Y substantially accelerated cell growth in vivo [16]. Interestingly, only cells transfected with the Cx43-T154Y mutant exhibited plasma membrane localization and allowed the transfer of the smaller molecules like neurobiotin, but not bigger molecules like Lucifer Yellow. The study by Moorby and Patel [17] provide direct evidence suggesting that growth regulation by Cx43 is independent of gap junction formation. They show that there was no correlation between the action of Cx43 mutants (S255A, S279A, and S282) on cell growth and Lucifer Yellow dye coupling in 3T3 A31 fibroblasts and that blockade of gap junction formation by either heptan-1-01 or culturing cells at low density had no effect on the ability of the Cx43 mutants to control cell growth. Moreover, wild-type Cx43 inhibited growth of Neuro2a cells independent of gap junction formation and the C-terminus of Cx43 that could not form gap junctions was as effective as the wild-type in suppressing the growth of Neuro2a cells. The second extracellular loop mutants of Cx43 (F199L, R202 E, and E205R) in another study were shown to inhibit cell growth despite their inability to form functional gap junction channels [18]. All these data suggest that certain mutant connexins exert dominant negative effects on connexin-regulated growth and that such effects are independent of the ability of GJIC.

Several studies show gap junction-dependent and -independent functions of connexins. Cx43 inhibited cardiomyocyte DNA synthesis irrespectively of cell–cell coupling; however, GJIC was required to reverse Cx43 inhibition of DNA synthesis by phosphorylation of Cx43 at S262, a phosphorylation site regulated by growth factor or protein kinase C [19]. In some cases, overexpression of connexins does not affect cell growth in vitro, but exerts the effect on tumor suppression. Bond et al. [13] found that Cx32-overexpressing C6 glioma cells did not have a