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Nontransformed cells can normalize gap junctional communication with transformed cells

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Abstract

We demonstrate that the Src kinase can augment gap junctional communication between cells derived from homozygous null Cx43 knockout mice. The total conductance between Src transformed cells was nearly twice that of nontransformed cells. In addition, the unitary conductance of the majority of single channel events between transformed cells was about 35% greater than that of nontransformed cells. Analysis showed that both nontransformed and transformed cells expressed at least two populations of channels, suggesting that Src increased junctional conductance by up-regulating one population and/or by increasing the unitary conductance of another population of channels. Interestingly, the conductance displayed by heterologous pairs of transformed and nontransformed cells resembled that of nontransformed cells. The majority of single channel events between heterologous pairs shifted back to lower conductances that were exhibited by nontransformed cells. Thus, nontransformed cells can effectively "normalize" the conductance of gap junction channels expressed by adjacent tumor cells.

Keywords: Gap junctions; Intercellular communication; Connexin; Src kinase; Cell junctions; Transformation; Heterologous; Growth control

Gap junctions form channels between adjacent cells exclusive of the extracellular space. Each gap junction channel is formed by 12 connexin subunits with each cell of a pair contributing six subunits. Connexins are an ancient protein family that has evolved into over 20 members in humans and mice [1,2].

Gap junction channels are permeable to a host of cytoplasmic solutes from monovalent cations and anions to second messengers and metabolites. The permeability of these molecules is governed by size and charge of a solute and the specific connexins comprising the gap junction channel [3–7]. This form of communication allows cells within a syncytium to function properly in a coordinated fashion [8–10].

At the organism level the critical role of connexins is evidenced by deleterious phenotypes in connexin knockout mice. For example, both Cx43 and Cx45 are required for proper development and function of the heart [11]. The importance of connexins to human health is further underscored by many diseases that are associated with mutations that affect connexin function or protein expression [1,12].

Evidence indicates that connexins play important roles in cell growth control. In particular, experiments have identified Cx43 as a tumor suppressor gene [13–15]. It is of pivotal interest that Cx43 can be phosphorylated by the Src tyrosine kinase. Moreover, this event

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reduces intercellular communication as revealed by dye transfer mediated by Cx43 between transformed cells [16–18].

We have previously shown that Cx45 is expressed in nontransformed and Src transformed cells derived from Cx43 homozygous null knockout mice, while Cx43, Cx40, and Cx30 are not [2]. In this study, we have investigated the effects of the Src tyrosine kinase on gap junctional communication between these cells. We examined the coupling between homologous pairs of transformed or nontransformed cells, as well as between heterologous pairs consisting of transformed and nontransformed cells.

A unitary conductance of about 28 pS was most frequently observed between homologous pairs of nontransformed cells, with less frequent events at 38 pS. Homologous pairs of Src transformed cells displayed a total conductance greater than nontransformed cells, with an increased occurrence of the 38 pS channel population. Heterologous cell pairs of transformed and nontransformed cells displayed reduced junctional coupling relative to homologous pairs, and the frequencies of unitary conductance resembled those of nontransformed cells at 28 pS.

These data demonstrate that neoplastic transformation can increase gap junctional communication between cells while heterologous coupling to nontransformed neighbors was reduced. However, the data also indicate that normal cells can modulate the gap junctional communication of adjacent tumor cells, particularly at the level of unitary conductance. These results may be applicable to in vivo situations where tumor cells meet surrounding tissue during growth and invasion.

Materials and methods

Cell culture. Nontransformed and v-Src transformed cells from homozygous null connexin 43 knockout mice (KoA and KoASrc) were used as described [2]. For all analyses, cells were maintained in DMEM supplemented with 10% FBS at 37 °C in 100% humidity. Src transformed cells were identified in coculture by staining with DiI or DiD as described, while nontransformed cells were stained with cell tracker green (5-chloromethyl-fluorescein diacetate; Molecular Probes) as described [2].

Evaluation of gap junctional communication. Electrophysiological measurements were performed on cell pairs cultured for 1–3 days. A dual voltage-clamp method and whole-cell recording was used to to control the membrane potential of both cells and to measure currents [3]. During experiments, cells were bathed in a solution containing: 110 mM CsCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Hepes (pH 7.4). The patch pipettes were filled with solution containing: 110 mM CsCl, 0.1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 3 mM EGTA, and 10 mM Hepes (pH 7.2). Data acquisition and analysis was performed with pClamp 8 software [19]. Curve fitting and statistical analysis were done with SigmaPlot and SigmaStat, respectively (Jandel Scientific). Results are presented as means ± SD.

Detection of connexin gene expression. Connexin mRNA expression was analyzed by RT-PCR as described [2]. Briefly, RNA was extracted from cells by the phenol–chloroform–isoamyl alcohol method, pretreated with DNase, and 200 ng was reverse transcribed in a thermal cycler (Perkin-Elmer, Norwalk, CT) using SuperScript II (Invitrogen #18064-014) and oligo(dT) primers according to manufacturer's instructions for 1 h at 42 °C in a 20 μl reaction. 0.2 μl of resulting cDNA was amplified with *Taq* polymerase and one set of oligonucleotide primers. Samples were denatured for 5 min at 95 °C and then amplified for 30 cycles at 94 °C for 45 s, 58 °C for 1 min, and 72 °C for 1 min. Two microliter aliquots from each PCR sample were then analyzed by agarose gel electrophoresis. Forward and reverse primer sequences were was follows: for Cx26 (5′-AGGAAGGTGCCACTGAGAAA, 5′-ACGAGACGCTTCCAGTTTGT), Cx30 (5′-GGTACCTTCTACTAATTAGCTTGG, 5′-AGGTGGTACCCATTGTAGAGGAAG), Cx32 (5′-CCATAAGTCAGGTGTAAAGGAGC, 5′-AGATAAGCTGCAGGGACCATAGG), Cx36 (5′-GTAGGGGAGACGGTGTACGA, 5′-TCGAAACACCACTTGGATGA), Cx37 (5′-CACACCCACCCTGATCTACC, 5′-ACCCCTACCACCAACATGAA), Cx40 (5′-CTGTCCCCACCCAGTCAACT, 5′-CCGTTTGTCACTATGGTAGC),Cx43 (5′-CCCCACTCTCACCTATGTCTC, 5′-ACTTTTGCCGCCTAGCTATCC), Cx45 (5′-TTCCAAGTCCACCCATTTTAT, 5′-ATCGTTCCTGAGCCATTCTGA), and GAPDH (5′-AATGCATCCTGCACCACCAA, 5′-GTAGCCATATTCATTGTCATA). Superscript II was omitted from some cDNA preparations to rule out false-positive reactions. RNA from brain tissue (cortex and cerebellum) isolated from an adult CD-1 mouse was used as a positive control.

Results

We have previously shown that nontransformed and Src transformed cells from Cx43 knockout mice express low levels of Cx45 [2]. We have verified this by RT-PCR with primers specific for Cx26, Cx30, Cx32, Cx36, Cx37, Cx40, Cx43, and Cx45. As shown in Fig. 1, both cell

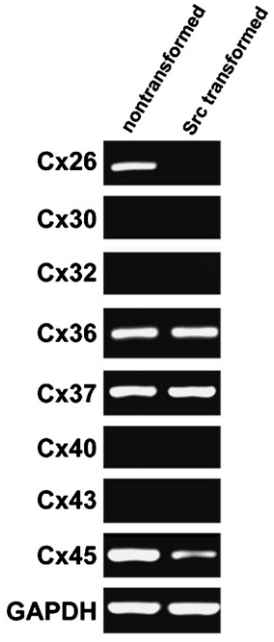

Fig. 1. Analysis of connexin mRNA. Expression of mRNA encoding several connexins was examined by RT-PCR. mRNA encoding Cx36, Cx37, and Cx45 was detected in both cell types, while Cx26 was only detected in nontransformed cells. Cx30, Cx32, Cx40, and Cx43 were not detected in either cell type.