Article Addendum

Connexin 43 gap junction plaque endocytosis implies molecular remodelling of ZO-1 and c-Src partners

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Gap junctions, through their constitutive proteins, connexins (Cx), are involved in several processes including regulation of cellular proliferation, tissue differentiation, homeostasis and neoplastic transformation. Internalization of the gap junction plaque to form annular gap junction is a dynamic process, which present similarities with endocytosis, and participates in the control of gap junction coupling. Cx43 exhibits dynamic trafficking that needs sequential implication of a large number of protein partners. We have recently shown that ZO-1 localized in both sides of the gap junction plaque was restricted to one side during internalization. The dissociation between ZO-1 and Cx43 particularly occurred on the face where c-Src specifically associated with Cx43 and was abnormally accelerated in response to a carcinogen. In this addendum we summarize and further discuss these results.

Gap junctions and connexins play an essential role in cell growth and differentiation and alteration of their expression has been associated with many diseases and is a typical feature of most tumor cells.1,2 A critical and long-standing question in gap junction biology associated with many diseases and is a typical feature of most tumor transformation. Internalization of the gap junction plaque to form gap junction plaque channel activity, tissue differentiation, homeostasis and neoplastic transformation. Internalization of the gap junction plaque to form annular gap junction is a dynamic process, which present similarities with endocytosis, and participates in the control of gap junction coupling. Cx43 exhibits dynamic trafficking that needs sequential implication of a large number of protein partners. We have recently shown that ZO-1 localized in both sides of the gap junction plaque was restricted to one side during internalization. The dissociation between ZO-1 and Cx43 particularly occurred on the face where c-Src specifically associated with Cx43 and was abnormally accelerated in response to a carcinogen. In this addendum we summarize and further discuss these results.

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Figure 1. (A) Carcinogen exposure induces gap junction plaque internalization that is associated with the increased interaction of c-Src with Cx43, decreased association between ZO-1. The three-dimensional Amira analysis of the altered interactions between Cx43 (green fluorescence) and ZO-1 (red fluorescence) during gap junction plaque internalization is summarized in upper panels. The lower panels illustrate the modified association between c-Src (red fluorescence) and Cx43 (green fluorescence) during the same time-period. Panel A5 represents the internal annular gap junction degradation of ZO-1 and panel A10 illustrates the disappearance of c-Src from this structure at the same period. (B) Identification of the different phases of gap junction plaque endocytosis analyzed by meaning of Cx43 tagged with green fluorescent protein (GFP). Left: Cx43-GFP gap junction plaque observed between two adjacent cells. Middle: invagination of the structure within one cell. Right: formation of an annular gap junction, which is present at the beginning of the endocytosis phase near the plasma membrane (arrow) and afterwards in the nuclear region around the nucleus (arrowhead). The inset represents a degradative form of annular gap junction. (C) Schematic representation of the molecular mechanisms by which ZO-1 and c-Src interact with Cx43 during gap junction plaque endocytosis. In control cells, Cx43 is associated with ZO-1. After HCH exposure, activated c-Src bind to Cx43 and induced Cx43 phosphorylation on tyrosine residues as suggested.30 These modifications could be involved in Cx43/ZO-1 dissociation in the gap junction side that binds c-Src, and induce gap junction internalization on the side of the gap junction plaque in which Cx43/ZO-1 association was probably affected in concert with other Cx43 protein partners (clathrin, actin...). Then annular gap junctions that contain ZO-1 inside and c-Src outside of the vesicle are degraded during annular gap junction trafficking from the plasma membrane to lysosomal area close to nuclei giving rise to disappearance of both proteins from their original position.
There is now evidence that modified interaction with other Cx protein partners could reduce Cx43/ZO-1 association and consequently gap junctional intercellular communication. Indeed by interacting with Cx43 the tyrosine c-Src kinase is involved in both, the dissociation of the Cx43/ZO-1 complex, and the downregulation of gap junctional cell-cell communication.15,23,24 Such a process has been clearly characterized after intracellular acidosis in astrocytes by ischemia or hypoxia.15,25 However, the molecular interactions between Cx43 and these two partners, which could drive Cx43 endocytosis in response to carcinogens,26–28 were unknown. In the course of a better understanding of the Cx43 partners involved in this process, we reported that c-Src-mediated dissociation of ZO-1 from one side of the plaque initiates gap junction endocytic internalization, and that this process can be markedly amplified in response to HCH, a non genomic carcinogen20 (Fig. 1A). We further demonstrated that the modification of the Cx43/c-Src/ZO-1 complex was subsequent to a rapid recruitment of c-Src to the plasma membrane, activation of c-Src, and efficient inhibition of gap junctional coupling. Since c-Src is known to be overexpressed and functionally upregulated in many types of human cancer,29 we speculated that c-Src, by altering the stabilization of the gap junction plaques, could conduce to severe disruption of cell-cell communication that may lead to tumor progression. Altogether our data demonstrate that membranous recruitment of c-Src not only induces closure of gap junction channel as previously reported,15,25 but can also stimulate gap junction internalization.

In conclusion, the results described in our recent study20 and discussed here allow postulating an innovating model of molecular reorganization (Fig. 1C) between Cx43 and two of its partners, c-Src and ZO-1, during gap junction plaque endocytosis. The possibility to apply this mechanistic endocytic model to other membranous proteins, unable to form large visualized structures, could be hypothesized.

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