Application Note: In Situ Porator – 003
November 2011

In situ electroporation for the examination of gap junctional communication; demonstration of a positive role for Stat3

Introduction

Gap junctions are plasma membrane channels that enable the passage of small molecules between the interiors of adjacent cells. A reduction in gap junctional, intercellular communication (GJIC) is believed to lead to an increase in cell proliferation. In fact, a number of oncogenes such as v-Src, v-Ras and others have been shown to interrupt junctional communication by phosphorylating connexin-43, one of the main components of gap junctions, both directly and indirectly, leading to GJIC suppression.

A cytoplasmic transcription factor which promotes cell division is the signal transducer and activator of transcription-3 (Stat3). Stat3 is activated by phosphorylation at a critical tyr-705, following stimulation by cytokines and receptor tyrosine kinases, as well as the non-receptor tyrosine kinase Src. Subsequent to phosphorylation, Stat3 dimerizes and migrates to the nucleus where it activates the transcription of genes involved in cell division and survival. The investigation of the role of Stat3 in neoplastic transformation gained momentum when it was found to be activated in a number of tumor cell lines and carcinomas. The fact that a constitutively active form of Stat3 alone is sufficient to induce transformation, points to an etiological role for Stat3 in neoplasia.

Despite the extensive literature on the effect of oncogenes upon GJIC, the effect of Stat3 is at present unknown.

Methods

We took advantage of the InSitu Porator to examine the effect of Stat3 upon gap junctional, intercellular communication (GJIC) in T51B rat liver epithelial cells, which have extensive GJIC. This setup can introduce large amounts of the tracer dye, Lucifer Yellow, into cells growing on a transparent electrode, in a non-traumatic manner. Following pulse application and introduction of Lucifer Yellow into the cells growing on the electrode by opening transient pores on the membrane, the migration of the dye by diffusion through gap junctions to adjacent, non-electroporated cells is microscopically observed under fluorescence illumination. Dye transfer can be precisely quantitated in this way, simultaneously and in a large number of cells, without any detectable disturbance to cellular metabolism. This approach was used to examine the role of Stat3 upon GJIC levels in rat liver epithelial cells.

Results

As shown in the Figure, T51B cells display a gradient of Lucifer Yellow fluorescence upon electroporation in the InSitu chamber, indicating that they have extensive communication (*c, d*). GJIC can then be quantitated by first identifying and counting the cells that the dye transferred into (marked by a dot), and dividing by the number of cells at the edge of the conductive coating that were loaded with the dye by electroporation. This can offer a numerical value for comparison purposes (*a,b*).

The Src oncogene is known to downregulate gap junctional communication through a variety of mechanisms including activation of the Ras/Raf/Mek/Erk pathway. Results have shown that Ras inhibition in Src-transformed cells restores GJIC. Since Stat3 is also activated by, and is required for transformation by vSrc, we used T51B cells transformed by activated Src as a model to explore the possible contribution of Stat3 in the suppression of gap junctional communication by Src. Stat3 was knocked-down through expression of siRNA with a retroviral vector. Unexpectedly, the results showed that Stat3 inhibition did ***not*** reinstate GJIC in T51B-Src cells, indicating that contrary to Ras, high Stat3 activity cannot be responsible for the lack of junctional communication in T51B-Src cells [1].

To further investigate whether Stat3 might, in fact, play a ***positive*** role upon gap junctional

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communication, we examined the effect of Stat3 inhibition in normal T51B cells which have extensive GJIC. Stat3 was downregulated through treatment with the pharmacological inhibitor CPA7, or shRNA expression, and GJIC examined. Interestingly, the results showed that Stat3 downregulation essentially ***abolished*** GJIC (*e-h*). The above data taken together reveal that, rather than increasing GJIC, Stat3 inhibition eliminates junctional permeability, indicating that Stat3 activity is ***required*** for gap junction function in normal epithelial cells.

Conclusions

The InSitu Porator is a powerful tool for the examination of intercellular, gap junctional communication. We unequivocally demonstrated that Stat3, although it is growth promoting and in an activated form can act as an oncogene, does not reduce gap junctional communication. In the contrary, Stat3 function is actually ***required*** for the maintenance of junctional permeability in normal epithelial cells.

[1] M. Geletu, L. Raptis *et al*. Stat3 activity is required for gap junctional permeability in normal epithelial cells and fibroblasts. DNA & Cell Biol. 28 (2009) 319327.

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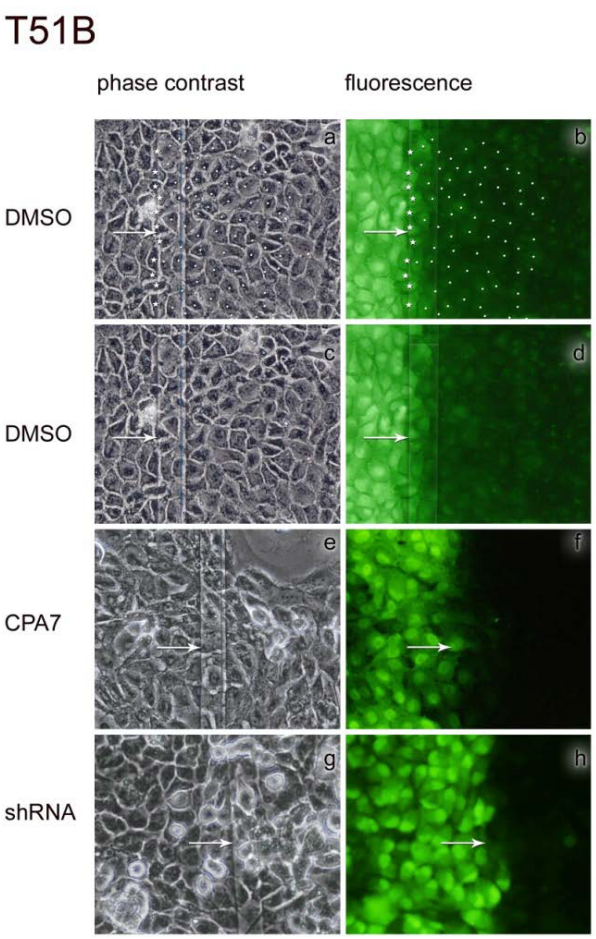

Arrows point to the edge of the electroporated area