Application Note: In Situ Porator – 002
November 2011

In situ electroporation of a peptide demonstrates inhibition of Erk activation by EGF

Introduction

The examination of protein interactions *in vivo* is often a necessary prelude to drug design. To this effect, protein complexes can be disrupted through the introduction of peptides corresponding to the proteins' point(s) of contact. We took advantage of electroporation, a fascinating cell membrane phenomenon, to inhibit signal transduction *in vivo*, using the InSitu Porator.

Growth factors such as the epidermal growth factor (EGF) stimulate cell proliferation by binding to, and activating membrane receptors with cytoplasmic tyrosine kinase domains. This induces receptor phosphorylation at distinct tyrosine residues. These become docking sites for a number of effector molecules, which are recruited to specific receptors through modules termed Src-homology 2 (SH2) domains. One such effector is Grb2, which binds to the receptors of growth factors such as EGF. This activates the Ras/Raf/Mek/Erk cascade, which is central to the mitogenic response triggered by many growth factors. To examine the role of the SH2 domain of Grb2 in EGF signalling, we determined the functional consequences of disrupting the association of Grb2 with the EGF receptor *in vivo*, by introducing a peptide (PVPE-Y^{p}–INQS) that can selectively bind this domain, into mouse NIH3T3 fibroblasts. To increase stability, phosphotyrosine was replaced by phosphono-methyl-phenylalanine. Following EGF stimulation, the phosphorylation, i.e. activation of the downstream target, Erk1/2 was assessed, by probing with a phospho-specific antibody.

Method

Mouse NIH3T3 fibroblasts were plated in InSitu Porator chambers. When confluent, cells were serum-starved overnight and the peptide was introduced by electroporation. Cells were stimulated with EGF for 10 minutes, then fixed and probed for phospho-Erk with a specific antibody.

As shown in the Figure, electroporation of this peptide totally inhibited EGF-induced Erk activation (upper panel, square bracket). This inhibition was uniform across the cell layer, indicating that *in situ* electroporation can introduce the material into essentially 100% of the cells treated. It is especially noteworthy that this inhibition ***extends into 3-4 rows of the adjacent, non-electroporated cells*** growing on the nonconductive part of the slide (upper panel, squiggly bracket), likely due to movement of the 1123 Da peptide through gap junctions. This finding constitutes compelling evidence that the observed inhibition must be due to the peptide, rather than an artifact of electroporation; these cells are not electroporated and they simply acquired the peptide via gap junctions from their neighbours. At the same time, as shown by phase contrast microscopy (lower panel), there was no alteration in the morphology of the electroporated cells, suggesting that the Erk inhibition observed is specific, and not due to toxic action.

As a control, the phenylalanine-containing peptide had no effect upon Erk activation. In contrast, the Grb2-SH2 binding peptide had little effect in inhibiting Erk activity triggered by the Hepatocyte Growth factor (HGF) in NIH3T3 cells expressing the HGF receptor through transfection, or in human A549 cells which naturally express this receptor [1].

The use of the *InSitu* Porator offers the opportunity to examine, in conjunction with gene product activity, cellular morphology and possible toxic effects. This approach can demonstrate the specificity of action of the Grb2-SH2 binding peptide, as well as examine the distribution of signal inhibition across the cell layer.

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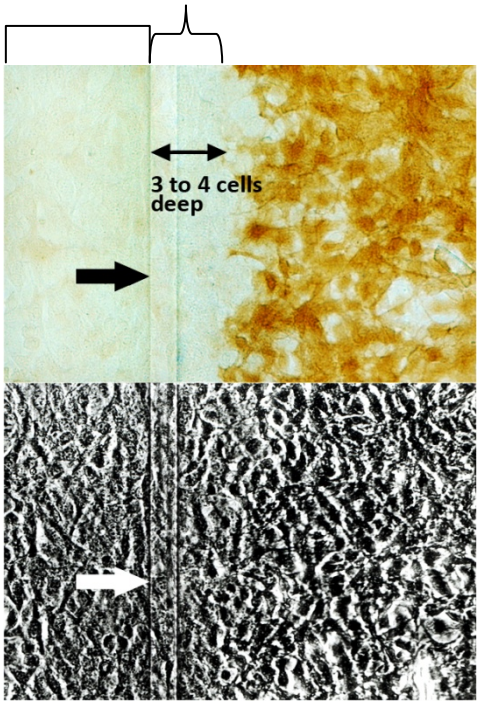
If the cells do have gap junctions, then the transfer of the peptide to the neighbouring, non-electroporated cells can give an even more powerful answer about the degree of signal inhibition. Currently, there is no other way to introduce molecules into cells without treating the cells themselves!
Contrary to other delivery techniques such as introduction in the form of fusion peptides where peptide penetration is relatively slow, *in situ* electroporation can achieve the instant introduction of large amounts of material into cells simultaneously, which facilitates kinetic studies of receptor activation.

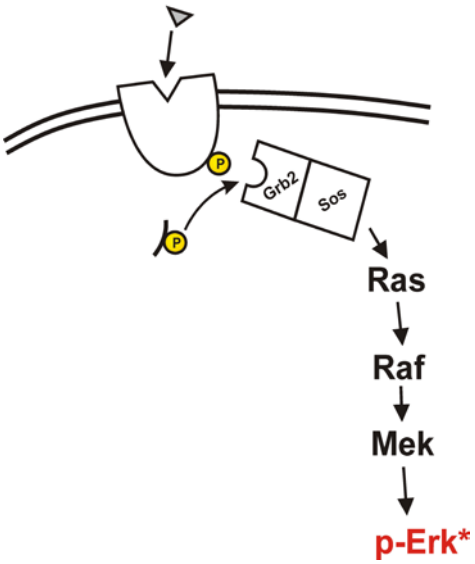


Arrowhead points to the edge of the electroporated area.

Conclusion

The introduction of peptides to interrupt signalling pathways using the InSitu Porator is a powerful approach for the *in vivo* assessment of the relevance of *in vitro* interactions. An essentially complete and specific inhibition of EGF-triggered, Erk activation can be achieved through electroporation of this peptide.
The stepwise dissection of signalling cascades is essential for the understanding of proliferative pathways, and the examination of the potential of different peptides to inhibit a specific pathway is the first important step in the development of peptidomimetic drugs, for the rational treatment of neoplasia.

[1] L. Raptis *et al*. Specific inhibition of Growth Factor-stimulated ERK1/2 activation in intact cells by electroporation of a Grb2-SH2 binding peptide. Cell Growth and Differentiation, 11 (2000) 293-303.



The peptide blocks the Grb2-SH2 domain and this inhibits activation of Erk.

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