

**P006** Proteinase-activated receptor-2 (PAR-2)-mediated phosphorylation of p65 NF $\kappa$ B in keratinocytes

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Proteinase-activated receptor-2 (PAR-2) is a novel G-protein coupled receptor which is activated by trypsin and synthetic ligands including 2furoyl-LIGKV-OH (2f-LIGKV-OH). PAR-2 has been implicated in several pathological conditions and such effects could be mediated via nuclear factor kappa B (NF $\kappa$ B) pathway. In this study we sought to examine the mechanisms by which PAR-2 utilises to mediate phosphorylation of p65 NF $\kappa$ B, which is a vital means of transactivation modulation.

Both trypsin and 2f-LIGKV-OH stimulated a time-dependent increase in p65 phosphorylation in NCTC-2544 cells stably expressing PAR-2 (clone G). By using adenoviral constructs encoding dominant negative inhibitory kappa B kinase (IKK) $\alpha$  and IKK $\beta$ , it was found that IKK $\beta$  plays a predominant role in regulating p65 phosphorylation in response to PAR-2 stimulation whilst IKK $\alpha$  was without effect. Furthermore, pre-treatment of clone G cells with the PKC inhibitors, GF 109203X and rottlerin, also largely abolished PAR-2-stimulated p65 phosphorylation. Finally, by using a novel G $\alpha_{q/11}$  inhibitor, YM 254890, we showed that this G protein subtype appears to partially regulate PAR-2-stimulated p65 phosphorylation and thus NF $\kappa$ B reporter activity. Similar findings were obtained in the primary keratinocytes, in that p65 phosphorylation mediated by PAR-2 was also found to be dependent on IKK $\beta$ , PKC and G $\alpha_{q/11}$ . Thus, this study has delineated the pathway of G $\alpha_{q/11}$ /PKC/IKK $\beta$  by which PAR-2 utilises to stimulate p65 phosphorylation in keratinocytes.