Control/Tracking Number: 06-A-2514-ARVO
Activity: Abstract
Current Date/Time: 12/2/2005 2:38:35 PM

Transglutaminase-2 Mediates p65 Translocation From Cytoplasm To Nucleus In The UVB-activated NF-_B Pathway

Author Block *L.M. Tong[1,2], Z.Chen[1], R.M. Corrales[1], X.P. Wang[1], C.S. De Paiva[1], D.Q. Li[1], S.C. Pflugfelder[1].* [1]Ocular Surface Center, Dept of Ophthalmology, Cullen Eye Institute, Baylor College of Medicine, Houston, TX; [2]Singapore National Eye Center, Singapore Eye Research Institute, Singapore.

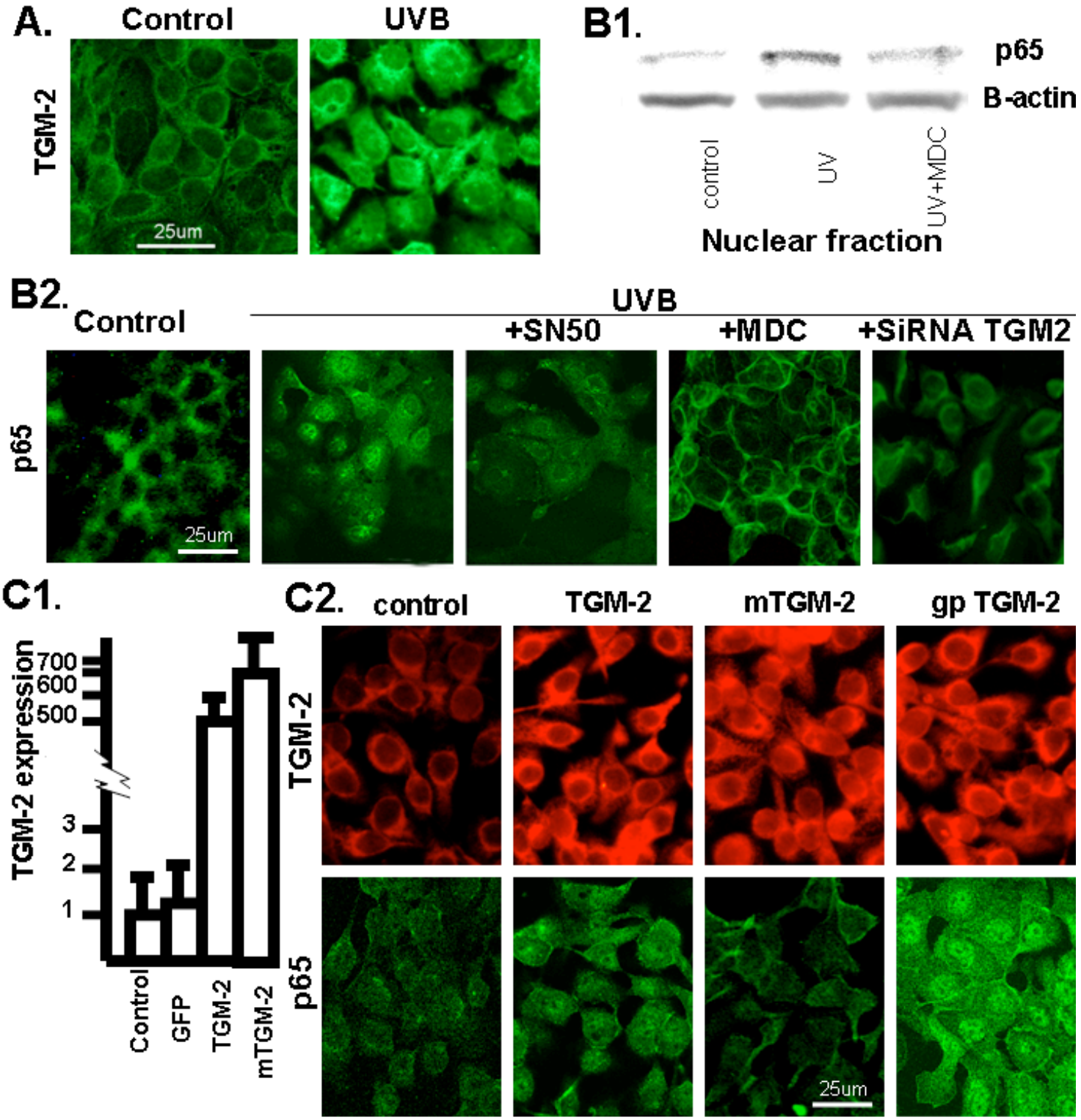
Purpose: Ultraviolet radiation (UVB) is a known stimulus for the activation of nuclear factor (NF-_B) in human corneal epithelial cells. The triggering mechanism for the NF-_B pathway is not known. We aimed to investigate whether transglutaminase (TGM)-2 mediates p65 cytoplasmic-nuclear translocation, a key step in NF-_B activation.
Methods: A human corneal epithelial cell line (T-HCEC) was irradiated by a uniform single dose (20 mJ/cm^2) of UVB with or without SN-50 or mono-dansyl cadaverine (MDC) pretreatment. Immunofluorescent staining (IF) and Western blot were performed using antibodies against p65 and TGM-2. IF with laser scanning confocal microscopy was used to assess p65 intra-cellular localization. A peptide-based non-covalent carrier system (Chariot^{TM}) was used to deliver guinea pig liver TGM-2 protein into T-HCEC without morphological evidence of toxicity. Short interfering RNA (RNAi-TGM) was used to silence TGM-2 gene expression. Biological TGM activity was assessed by a fluorescein-cadaverine uptake assay.
Results: UVB induced an increase in TGM-2 protein level and trans-amidation activity and also induced the translocation of p65 protein from cytoplasm to nucleus (IF) after 4 hours in T-HCEC. This translocation was verified by Western blots showing that the p65 immuno-reactivity increased in the nuclear fraction of the cell lysates of UVB-exposed cells relative to control. This UVB-induced p65 translocation was inhibited by SN-50 peptide, a cell-permeable chemical that inhibits nuclear translocation of active NF-_B, and by MDC, a competitive TGM inhibitor. We observed in non-irradiated cells that recombinant human TNF-_ induced the p65 nuclear translocation, which was also inhibited by MDC or RNAi-TGM. Furthermore, the exogenous TGM-2 protein introduced into non-irradiated T-HCEC by the Chariot system triggered p65 nuclear translocation, whereas cells incubated with either TGM-2 or Chariot reagent alone were not demonstrated to have p65 nuclear translocation.
Conclusions: Our findings demonstrate that increase in TGM-2 protein mediates p65

nuclear translocation in the UVB-activated NF-_B pathway in human corneal epithelial cells. TGM-2 could be a potential therapeutic target in ocular surface inflammatory diseases where NF-_B activation is a common mechanism.

Author Disclosure Block: L.M. Tong, None; **Z. Chen**, None; **R.M. Corrales**, None; **X.P. Wang**, None; **C.S. De Paiva**, None; **D.Q. Li**, None; **S.C. Pflugfelder**, None.

Reviewing Codes (Complete): 152 corneal inflammation – CO
Presentation Preference (Complete): Paper #1, Poster #2
Keyword (Complete): 477 cornea: epithelium ; 699 signal transduction ; 710 stress response
VSS, International, Clinical Trial, Newsworthy (Complete):
Yes: True
***Human Controlled Clinical Trial**: No
***Newsworthy**: Yes
Public: True
Researchers in Other Disciplines: True
Clinicians: True

Support (Complete):
***Support**: EY11915 (SCP), EY014553 (DQL), Agency for Science Technology and Research, Singapore, unrestricted Grant :Research to Prevent Blindness, Oshman Foundation, William Stamps Farish Fund



Transglutaminase (TGM) -2 induces NFκB/p65 cytosol-nuclear translocation in human corneal epithelial cells. UVB up-regulated TGM-2 protein (**A**. immunofluorescent staining). UVB stimulated p65 translocation to nuclei detected by Western blot (**B1**) and immunostaining, while SN-50, a soluble peptide inhibitor of NF-κB, mono-dansyl cadaverine (MDC), a transglutaminase inhibitor, or SiRNA against TGM-2 blocked this translocation (**B2**). Transfection with plasmids expressing active or cys277 to ser mutant (mTGM-2) increased the TGM-2 transcripts compared to GFP expression or un-transfected controls ($p<0.0001$) by real time RT-PCR (**C1**). The over-expressed TGM-2 but not mTGM-2 protein, as well as the exogenous guinea pig (gp) TGM-2 via Chariot™ delivery, stimulated the p65 nuclear translocation (**C2**).