GW23-e1253

EFFECTS OF XERODERMA PIGMENTOSUM D GENE ON PROLIFERATION INDUCED BY INTERLEUKIN-6 IN VASCULAR SMOOTH MUSCLE CELLS

doi:10.1136/heartjnl-2012-302920a.90

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Objectives Excessive proliferation vascular smooth muscle cells (VSMC) can promote the development of atherosclerosis. And, in the development of atherosclerosis, interleukin-6 (IL-6) enhance excessive proliferation of VSMC.As studies have showed, up-regulation of xeroderma pigmentosum D(XPD) gene could induce apoptosis of a variety of cells. However, it has not been reported whether there is any effect of XPD on proliferation and apoptosis of VSMC. To investigate effects of XPD on proliferation induced by IL-6 in human VSMC and its association with atherosclerosis, this study was carried out.

Methods

1. Recombinant plasmid pEGFP-N2/XPD and vacant vector plasmid pEGFP-N2 were transfected stably into VSMC by liposome, and then these cells were incubated with IL-6 at a 100 U/mL concentration for 48 h. The experiments were divided into six groups: blank control group; pEGFP-N2 group; pEGFP-N2/XPD group; IL-6 group; IL-6+pEGFP-N2 group; IL-6+pEGFP-N2/XPD group.
2. The expression of green fluorescent protein was observed through fluorescence microscopy.
3. The cell growth was detected by MTT.
4. The cell cycle and apoptosis rate were examined with flow cytometry.
5. Through RT-PCR and Western blotting, the expression levels of XPD, Bcl-2, Bax and wild type P53 (wt-P53) were detected.

Results

1. By fluorescence microscopy, green fluorescences were observed in the cells transfected with pEGFP-N2/XPD or pEGFP-N2, indicating that the plasmids were transfected successfully.
2. MTT results showed that the transfection of pEGFP-N2/XPD inhibited the cell growth ($p<0.05$), and reduced the positive effects of IL-6 on VSMC growth ($p<0.05$).
3. Flow cytometry results showed that the transfection of pEGFP-N2/XPD increased the apoptosis rate of VSMC ($p<0.01$) and the cell amounts of G0/G1 phase ($p<0.05$), decreased the cell amounts of S phase ($p<0.05$), and reduced the effects that IL-6 decreased the apoptosis rate of VSMC and the cell amounts of G0/G1 phase, increased the cell amounts of S phase ($p<0.01$). 4. RT-PCR results and western blotting results showed that the transfection of pEGFP-N2/XPD increased the expression of XPD, Bax and wt-P53 ($p<0.05$ or $p<0.01$), decreased the expression of Bcl-2 ($p<0.05$ or $p<0.01$), and reduced the effects that IL-6 decreased the expression of Bax and wt-P53, increased the expression of Bcl-2($p<0.05$ or $p<0.01$).

Conclusions XPD gene can inhibit VSMC proliferation, promote VSMC apoptosis, and reduce the effects that IL-6 promotes VSMC proliferation and inhibits VSMC apoptosis. Therefore, XPD gene is likely to be potential molecular target for treatment of atherosclerosis.