論 文 要 旨

Low grade inflammation inhibits VEGF induced HUVECs migration in p53 dependent manner

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OBJECTIVES:

The study was conducted to explore the role of low grade inflammation in growth factor induced angiogenesis.

METHODS:

We performed Wound healing assay (scratch assay) in HUVE cells using tumor necrosis factor alfa (TNFα) and vascular endothelial growth factor A (VEGF) respectively. We used western blotting, immunofluorescence and RT-PCR to track the status of different proteins involved in the crosstalk.

RESULTS:

In scratch assay, we found that low dose TNFa pretreatment decreased the VEGF induced HUVECs migration. Western blotting and immunofluorescence revealed that P53 was upregulated and translocated to the nucleus in TNFa pretreated and VEGF co-incubated groups. When P53 was silenced, TNFa could not decrease the VEGF induced cell migration which confirmed the role of P53 to inhibit cell migration. Western blotting showed that TNFa pretreated and VEGF co-incubated groups which had increased P53 also had decrease in Id1. Immunofluorescence study showed that in P53 silenced groups, Id1 was freely shuttled out of the nucleus because P53 inhibits the cytoplasmic shuttling of Id1. We checked the status of two b-integrins in our culture. We found that there was no difference in b1-integrin status in TNFa and VEGF co-incubated groups compared to their control counterparts. Loss of p53 have been reported to increase lamillipodia formation and recruitment of $\alpha v\beta_3$-integrin to lamellipodia is important in endothelial cell migration. When Id1 was silenced, we found that protein level of b3-integrin was decreased as seen by western blotting.

CONCLUSIONS:

High doses of pro-inflammatory cytokines have been reported to induce apoptosis in cells through upregulation of lethal amount of tumor suppressor protein 53 (P53). Under physiological conditions also the pro-inflammatory cytokines are being released in low doses and are actively involved in cell signaling pathways. We studied the effects of low grade inflammation in growth factor induced angiogenesis using tumor necrosis factor alfa (TNFα) and vascular endothelial growth factor A (VEGF) respectively. We found that low dose of TNFα can inhibit VEGF induced angiogenesis in human umbilical vein endothelial cells (HUVECs). Low dose of TNFα induces mild upregulation and moreover nuclear localization of P53 which causes decrease in inhibitor of DNA binding-1 (Id1) expression and shuttling to the cytoplasm. In absence of Id1, HUVECs fail to upregulate β_3-integrin and cell migration is decreased. We reported a signaling pathway for low dose of TNFα induced inhibition of VEGF induced HUVECs migration.