論 文 要 旨

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

（小規模の炎症反応は、VEGF で誘導されるヒト血管内皮細胞の遊走能を p53 分子依存性に抑制する。）

Sushil Panta

【序論及び目的】

The signaling pathway for endothelial cell migration involve inflammatory factor like TNFα and angiogenic factor like VEGF. Once TNFα is secreted in response to toxins, its first target is the endothelium. VEGF is a major factor for endothelial cell migration and its understanding will be useful to control the different pathological conditions. P53 is a hub for the cell signaling pathway and relays the respective signals to their targets. Cells must acquire lethal level of P53 faster to undergo apoptosis. Since P53 has been reported to attenuate cancer cell migration, we hypothesize that TNFα induced mild upregulation of P53 must be involved in controlling cell migration induced by VEGF. Our aim in this study is to explore the role of low grade inflammation in growth factor induced angiogenesis.

【材料及び方法】

HUVECs were incubated in EBM-2 basal medium containing 0.2% FBS for experimental purposes. Wound healing assay and transwell migration assay were performed to observe and quantify cell migration. Western blotting, ELISA and quantitative real-time RT-PCR was used to quantify different proteins and their mRNA levels respectively. The importance of the proteins in cell migration were established by silencing the proteins of interests also. Localization of the proteins were observed by immunofluorescence imaging. Data were presented as means ± S.D. of several determinations. Differences between groups were tested for significance using Student's t-test and $P \leq 0.05$ was considered as statistically significant. In these assays, similar results were obtained in at least three independent experiments.

【結　果】

Pretreatment of HUVECs with low dose TNFα and co-incubation with VEGF resulted into reduced migration compared to VEGF treatment only. MTT assay and VEGFR ELISA revealed that the decrease in the HUVECs migration by TNFα pretreatment was not due to the decrease in cell number or VEGF receptors in TNF treated groups. TNFα and VEGF co-incubated cells showed mild elevation and nuclear localization of P53 compared to TNFα and/or VEGF only treated groups. RT-PCR analysis showed that pretreatment with TNFα did not make much difference in p53 mRNA level. TNFα pretreatment could not inhibit the VEGF induced migration in P53 silenced group. TNFα pretreatment inhibited the shuttling of Id1 from the nucleus to the cytoplasm while VEGF treatment released it to the cytoplasm. In P53 silenced groups, distribution of Id1 in cytoplasm was evident. The upregulation of P53 by the concerted effort of TNFα and VEGF was followed by downregulation of Id-1. Compared to scramble, Id1 level was drastically upregulated in P53 silenced group. TNFα pretreatment and VEGF co-incubation made no significant difference between β_1-integrin levels. $\alpha_5\beta_3$–integrin is an important one regarding its ability to bind to wide variety of plasma proteins and matrix and expression on angiogenic vessels. Id-1 have been reported to control $\alpha_5\beta_3$-integrins. We silenced Id1 in HUVECs and checked the level of β_3-integrin. Compared to scramble, Id1 silenced cells had significantly decreased level of β_3-integrin.

【結論及び考察】

Low grade acute inflammation have been used to treat bladder cancer in the past. We used TNFα in our experiment because classical features of inflammation in endothelial cells can be achieved by treating the cells with TNFα. TNFα can be pro-tumorogenic or tumoricidal agent depending on its local tissue concentration. We found that in HUVECs low dose TNFα exerts the anti-migratory activity against VEGF. We focused on inhibition of cell migration by a nonlethal and mild upregulation of p53 for which we used much lower dose of TNFα. TNFα pretreated and VEGF co-incubated group always had mild increase of P53 levels. Comparison of control versus P53 silenced group showed that P53 is an important conductor of low grade inflammation triggered antiangiogenic response. We choose Id1 as a potential candidate to be investigated in P53 controlled cell migration. TNFα pretreatment downregulated the VEGF induced Id1. In P53 silenced group, TNFα pretreatment was unable to downregulate the Id1 induced by VEGF treatment. Recruitment of the downstream target of Id1 i.e. $\alpha v\beta_3$-integrin to lamellipodia is important in endothelial cell migration. β_3-integrin was significantly downregulated in Id1 silenced HUVECs. In summary, the anti-angiogenic signal of TNFα is relayed by P53 to suppress Id1 activity. Suppression of Id1 activity caused downregulation of β_3-integrin that decreased the HUVECs ability to migrate.

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