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Signalling pathways in angiogenesis

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Keywords

Cell cycle, endothelium, growth factors, p70 S6 kinase

Context

During angiogenesis, endothelial cell (EC) proliferation is a critical event that contributes to development of the invasive pannus in rheumatoid arthritis (RA), as well as playing an important role in other angiogenesis-dependent pathological conditions. Proliferation in response to growth factors and mitogens requires an increase in the rate of protein synthesis in order to enter the G1 phase, and subsequently to proceed to S phase. The Ras-p42/p44 mitogen activated protein kinase (MAPK) and the phosphatidylinositol 3-kinase (PI3K) signalling cascades play key roles in mediating this increase in protein synthesis. Two downstream signalling pathways have been described for PI3K. One pathway involves p70 S6 kinase. This is a serine/threonine kinase which controls multiple phosphorylation of the 40S ribosomal protein S6 and is activated by many mitogenic stimuli, including growth factors and cytokines. p70 S6 kinase can be distinguished from the other signalling pathway downstream of PI3K, Akt-GSK3, by its sensitivity to rapamycin, which is a potent inhibitor of p70 S6 kinase, preventing its phosphorylation and activation. This study analysed the contribution of PI3K-p70 S6 kinase in the control of vascular endothelial cell growth.

Significant findings

Synthesis of DNA in 1G11 ECs, stimulated with foetal calf serum (FCS), was inhibited by pre-incubation of cells with wortmannin or LY294002. Treatment with wortmannin or LY294002 prevented PI3K activation, as measured by the phosphorylation state of PI3K targets Akt and p70 S6 kinase. Pre-incubation of 1G11 cells with PD98059 or rapamycin also inhibited the mitogenic effect of FCS. Inhibition of p70 S6 kinase activity by rapamycin was observed, whereas p42/p44 MAPK activation was unaffected. Comparable inhibition of FCS-stimulated thymidine incorporation was observed in HUVECs and LIBE cells, but not in CCL39 fibroblasts. Rapamycin blocked mitogenicity at all the times assayed, indicating that in vascular ECs, rapamycin blocks cells in G1 phase, rather than delaying entry into the cell cycle. The effect of rapamycin on thymidine incorporation correlated with protein synthesis inhibition, suggesting that the block of mitogenesis induced by rapamycin is due to the inhibition of serum-stimulated protein synthesis. Treatment of quiescent 1G11 cells with 20% FCS caused an induction of two proteins expressed in G1, cyclin D1 and p21, which was completely abrogated by treatment with rapamycin. In contrast, degradation of the cyclin-dependent kinase inhibitor p27 and the synthesis of an early-gene protein, MKP1, were not affected by treatment with rapamycin. To evaluate

whether the effects of rapamycin are driven by p70 S6 kinase inhibition, 1G11 cells stably expressing either wild type or a rapamycin-resistant mutant form of p70 S6 kinase were isolated. Rapamycin inhibited thymidine incorporation in clones overexpressing the wild-type form with the same dose response as in parental cells. In contrast, cells overexpressing the rapamycin-resistant form of p70 S6 kinase were resistant to inhibition.

Comments

EC proliferation and migration to form new blood vessels *in vivo* - angiogenesis - has been shown to play a key role under both normal physiological conditions and in the development of many diseases, including malignancies and RA. As an indicator of the emerging importance of this area, there were just 145 references in the National Library of Medicine's bibliographic database with the key words 'angiogenesis' or 'angiogenic' in the 1970s. This rose to 1004 in the 1980s, and 6907 in the 1990s. Angiogenesis has become a 'growth area'. The demonstration that at least two separate growth-signalling cascades are operating in vascular ECs, leading to activation of p42/p44 MAPK p70 S6 kinase, is another step towards increasing our understanding of the mechanisms involved in endothelial proliferation.

Methods

Incorporation of [^{3}H]-thymidine and [^{3}H]-leucine were measured respectively using the following cells: murine lung endothelial 1G11 cells, murine brain capillary endothelial LIBE cells, human umbilical vein ECs (HUVECs) and the Chinese hamster lung fibroblast line CCL39. Inhibition of kinase enzymes was achieved using wortmannin and LY294002 to block PI3K, PD98059 to inhibit MEK1 (the upstream activator of p42/p44 MAPK), and rapamycin to block p70 S6 kinase. Wild type and rapamycin-resistant p70 S6 kinase-expressing 1G11 cells were generated by transient transfection of BOSC23 cells with plasmids pBabe or pBabe-p70 S6 kinase (either wild type or a rapamycin-resistant mutant, with an amino-terminal Myc tag). Western blot analysis was used to assess p70 S6 kinase, Akt/phospho-Akt, p42MAPK, cell cycle proteins cyclin D1, p21 and p27, MAPK phosphatase 1 (MKP1) and Myc.

References

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