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Characterisation of a novel gene p73RhoGAP in angiogenesis.

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ABSTRACT

Angiogenesis is the formation of new blood vessels from pre-existing vessels. It is highly regulated in a normal physiological system. Dysregulated angiogenesis is associated with a a number of pathological states such as cancer and rheumatoid arthritis. Thus angiogenesis is proposed as a target for control of such diseases. The identification of novel genes involved in angiogenesis will improve the likelihood of the development of efficacious drugs.

To identify genes essential to angiogenesis, we used an *in vitro* model of capillary tube formation and a PCR based subtraction hybridisation approach for isolation of regulated genes. We identified 125 different genes including 96 known and 29 novel genes and of these, 84 genes were confirmed to be regulated during the formation of capillary tubes.

From the 125 isolated genes, one novel gene displayed characteristics suitable for further investigation. The gene, p73RhoGAP is a new member of the RhoGAP family. It is highly upregulated at 3 and 6 h in the tube formation process but only in an angiogenic milieu with little or no regulation seen under non-angiogenic conditions, and is restricted in its expression to vascular cells. Thus, we propose that p73RhoGAP is called VASGAP. Bioinformatics analysis of the protein sequence revealed the presence of a putative RhoGAP domain, indicating a potential role in the activation of RhoGTPases. Our study suggests that VASGAP displays RhoGAP activity for Rho but not Rac or Cdc42. RhoGTPase activity assays demonstrated that mutation of the conserved arginine at residue 82 (R82A) in the RhoGAP domain is crucial to the p73 effect on Rho. VASGAP localises to actin stress fibres.

To characterise the function of VASGAP, knockdown of the protein was achieved by adenovirus delivery of VASGAP antisense into EC. Such VASGAP depleted cells showed enhanced actin stress fibres and basal permeability consistent with alteration of Rho activity. Cell migration, proliferation and capillary tube formation were inhibited, and enhanced apoptosis was evident. In an *in vivo* model of angiogenesis, antisense of VASGAP inhibited blood vessel invasion.

Thus, the results suggest VASGAP is an important and novel modulator of angiogenesis and cell survival and displays many of the features worthy of a drug target.