PublisherInfo		
PublisherName	:	BioMed Central
PublisherLocation	:	London
PublisherImprintName	:	BioMed Central

Molecular fingerprinting during endothelial cell differentiation

ArticleInfo		
ArticleID	:	167
ArticleDOI	:	10.1186/ar-2000-66829
ArticleCitationID	:	66829
ArticleSequenceNumber	:	124
ArticleCategory	:	Paper Report
ArticleFirstPage	:	1
ArticleLastPage	:	3
ArticleHistory	:	RegistrationDate : 2000–8–9 OnlineDate : 2000–8–9
ArticleCopyright	:	Current Science Ltd2000
ArticleGrants	:	
ArticleContext	:	130753311

Oliver Distler,[Aff1]

Aff1 Center of Experimental Rheumatology, Zurich, Switzerland

Keywords

Angiogenesis, endothelial cells, GeneCalling, inflammation

Context

Angiogenesis plays an important role in the development and progression of a number of diseases, including rheumatoid arthritis (RA) and psoriasis. Understanding the molecular events that direct angiogenesis is important for the development of therapeutic strategies to inhibit or promote angiogenesis. To identify a transcriptional profile of endothelial cell differentiation into tubelike structures using amplification and GeneCalling.

Significant findings

Using GeneCalling and oligonucleotide poisoning, 90 distinct genes were identified that showed temporal changes in their expression pattern during tubule formation. In addition, 80 cDNA fragments were identified as novel or as corresponding to expressed sequence tags of unknown function. TaqMan analysis confirmed the differential expression of 67 of the genes identified by GeneCalling. Alterations in gene expression were grouped into four patterns. Group I (early transient expression) included changes in expression of IL-8, binding protein A1, plasminogen activator inhibitor-2, GRO-a and COX-2. Group II (delayed transient expression) consisted of the genes white protein homolog, fibroblast growth factor-16, ADAMTS-4 (aggrecanase-1), cathepsin B, podocalyxin-like protein and epithelial tyrosine kinase. Group III (stable induction) included placental growth factor, yLAT1, osteonidogen, MMP-9, CXCR4 and stanniocalcin precursor (STC). Unlike levels in the other groups, genes of group IV (rapid repression) declined rapidly from the initial value. This group included extracellular protein S1-5, axl, polo-like kinase and mesothelial keratin. The biological relevance of STC, podocalyxin, osteonidogen and ADAMTS-4 was additionally shown by *in situ* hybridization in different tumors as well as in a variety of human organs.

Comments

Using an elegant, novel, comprehensive mRNA profiling technique named GeneCalling, Kahn and co-workers identified 115 cDNA fragments that are differentially regulated during endothelial cell differentiation *in vitro*. Among these differentially expressed genes, several had not yet been implicated in angiogenesis. These findings may help to identify new molecular targets for therapeutic approaches; however, the study has limitations: biological relevance has been shown by *in situ* hybridization for only four newly identified genes; and the authors used three-dimensional collagen gels that require incubation with a distinct mixture of angiogenic stimuli that may not fully mimic new vessel formation *in vivo*.

Methods

Tube formation of human umbilical vein endothelial cells was assessed in three-dimensional collagen gels incubated with a mixture of angiogenic stimuli. RNA was extracted at different time points. After treatment with restriction enzyme pairs, cDNA was amplified using specific linkers and PCR products were visualized by the presence of the reporter dye FAM using multicolor laser excitation. GeneCalling (Curagen Corporation, New Haven) utilizes the restriction enzyme pair recognition site information and the cDNA fragment size to search for likely gene matches in public databases using statistical and mathematical criteria. GeneCalls were confirmed in competitive PCR reactions ("GeneCall poisoning"), quantitative RT-PCRs (TaqMan [Perkin Elmer Biosystems]) and in part by *in situ* hybridization on various tissues.

References

1. Kahn J, Mehraban F, Ingle G, Xin X, Bryant JE, Vehar G, Schoenfeld J, Grimaldi CJ, Peale F, Draksharapu A, Lewin DA, Gerritsen ME: Gene expression profiling in an *in vitro*model of angiogenesis. Am J Pathol . 2000, 156: 1887-1900.