HUMAN ARTERIAL SMOOTH MUSCLE CELLS IN CULTURE:
INVERSE RELATIONSHIP BETWEEN PROLIFERATION
AND EXPRESSION OF CONTRACTILE PROTEINS

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SUMMARY

Human arterial smooth muscle cells (hASMC) from explants of the inner media of uterine arteries were studied in secondary culture. We had previously found that these cells depend on exogenous platelet-derived growth factor (PDGF) for proliferation in vitro. Deprivation of the serum mitogen(s) by culture in plasma-derived serum or bovine serum albumin (BSA) caused a true growth arrest that was reversible upon reexposure to the mitogen(s). When added to serum-containing medium, heparin caused a reversible growth arrest which could be competed for by increasing concentrations of serum. In the current study we used a set of smooth muscle-specific actin and myosin antibodies to study the expression of contractile proteins in stress fibers under indirect immunofluorescence on hASMC in culture. Even in sparse culture, growth-arrested hASMC expressed stress fibers containing these actin and myosin epitopes. This was true irrespective of whether growth arrest was achieved by culture in media containing only BSA or a combination of heparin and whole blood serum. hASMC proliferating in whole blood serum in sparse culture did not express such stress fibers, as judged by immunofluorescent staining. This was true also for cells that were restimulated to proliferate in serum after a growth arrest. Utilizing a monoclonal antibody against a nuclear antigen expressed in proliferating human cells, we were able to demonstrate an inverse relationship between the expression of this antigen and the SMC-specific contractile proteins, respectively. Under these culture conditions, the reversible transition between dedifferentiated and differentiated hASMC was almost complete and terminated about 1 wk after the change in culture condition. We conclude that hASMC in vitro respond to exogenous PDGF by proliferation and dedifferentiation as a single population of cells. We also conclude that this modulation is reversible, because the cells become uniformly quiescent and differentiated when the mitogenic stimulus is blocked or removed.

Key words: smooth muscle cells; human; vascular; differentiation; cytoskeleton; actin; myosin.

INTRODUCTION

Increased arterial smooth muscle cell (ASMC) mass is typically found in the intimal lesions of advanced atherosclerosis as well as in the medial changes of arterial hypertension. The temporal sequence of events in human atherosclerosis can only be deduced from a few important observations of atheromatous tissue in humans. These are compatible with the concept that a reversible modulation between a differentiated (quiescent) (10,19,27) and a dedifferentiated (proliferative) (21,22) phenotype may occur among ASMC (possibly in response to external stimuli) during the atherosclerotic process. The mechanisms behind this process cannot, however, be studied in detail in man. Conclusions regarding possible mechanisms have instead been derived from extensive experimental studies of animal systems [reviewed in (24,26)]. The results of such studies may not be altogether relevant for man and need to be confirmed by, for example, in vitro studies of human ASMC.

We have previously isolated human ASMC (hASMC) and presented data on their growth control in culture (5). In summary, we found that the hASMC depended on exogenous platelet-derived growth factor (PDGF) for their proliferation in vitro. In plasma-derived serum...
(PDS) or in one percent bovine serum albumin (BSA), 
hASMC became growth-arrested within 2 d and remained 
quiescent for up to at least 7 d. This state was, however, 
reversible upon reexposure to serum. The mitogenic 
activity of serum was effectively counteracted by 
anti-PDGF IgG. Under no condition that we studied in 
vitro could we find any expression of PDGF mRNA in 
hASMC, as indicated by Northern blots using both A and B 
chain cDNA probes. When added to the medium, heparin 
inhibited the mitogenic effect of serum on hASMC in a 
dose-dependent, reversible and competitive way. Our 
results suggest that the effect of heparin is due to 
interference with the binding of PDGF to its cell surface 
receptor. This conclusion was based on the observation 
that hASMC expressed PDGF receptors on their surface 
when heparin was added to a serum-containing medium. 
In the absence of heparin, the receptor was down-
regulated in serum-containing media.

In this study we do not address the well-established 
differentiation that occurs in spite of the presence of 
mitogens through contact inhibition in postconfluent 
SMC cultures (3,23,25). Instead, the current study was 
aimed at investigating whether the mitogen-dependent 
and reversible transition between quiescent and prolifer-
ating hASMC in sparse culture was associated with a 
modulation in phenotype, as determined by the expres-
sion of SMC-specific contractile proteins in stress fibers 
at the level of the individual cell.

**MATERIALS AND METHODS**

**Materials.** The basal medium (BM) in this study was 
Waymouth’s MB 7521 medium containing 10° IU/liter 
penicillin, 100 g/ml streptomycin, 1 mM sodium 
pyruvate, and 4 mM L-glutamine. These components, as 
well as trypsin (no. 16-893) and Earle’s balanced salt 
solution without Ca++ and Mg++ (EBSS), were from 
Flow Laboratories (Irvine, Scotland). Culture flasks (80 
cm²) and petri dishes (10 cm²) came from Nunclon 
(Roskilde, Denmark) and fetal bovine serum (FBS) from 
Tissue Culture Services (Berkshire, UK). Human serum 
(HS) was obtained from healthy volunteers. Bovine serum 
albumin (Fraction V, no. A 4503) was provided by Sigma 
Chemical Company (St. Louis, MO). Heparin sodium 
powder (Heparin Sodium Pure) from swine mucosa was a 
gift from Leo Pharmaceutical Products (Ballerup, 
Denmark).

In the individual experiments, the BM was supplement-
ed with sera, BSA, or heparin as indicated.

**Antibodies.** A monoclonal antibody against SMC-
specific alpha and gamma actin isoforms (CGA7) has 
previously been characterized (9). CGA7 stains growth-
arrested, but not proliferating rat ASMC, and has been 
found to be a marker of SMC differentiation (23). A 
second monoclonal antibody against smooth, cardiac, 
and skeletal muscle alpha actin and smooth muscle 
gamma actin isoforms (HHF35) (28), as well as a third 
monoclonal that reacts exclusively with smooth muscle 
alpha actin (anti-asm-1) (27), was also used to stain 
SMC-specific actins. A rabbit antisem against SMC-
specific myosin (ASMM) was used to detect myosin-
containing stress fibers in SMC. ASMM antisem reacts with 
the heavy chain (200 kDa) of myosin in mature cells in 
vascular and visceral smooth muscle (18,19). The four an-
tibody preparations were known to react with human actin 
and myosin epitopes, respectively.

We used a monoclonal antibody to stain proliferating 
cells (PC) in culture. This PC antibody (Ki-67) 
(Dakopatts, Copenhagen, Denmark) specifically recog-
nizes an antigen expressed in the nuclear membrane of 
human cells in G1 (late), S, G2, and M phases of the cell 
cycle (8).

Fluorescein-(FITC) and rhodamine-(TRITC) conjugat-
ed rabbit antimouse immunoglobulins, as well as TRITC 
swine antirabbit immunoglobulins and nonimmune 
swine serum, were provided by Dakopatts (Copenhagen, 
Denmark). Para-phenylenediamine (PPD) was purchased 
from Fluka AG (Buchs, Switzerland).

**Cells and cell culture conditions.** Primary cultures of 
hASMC from the inner media of human uterine arteries 
were established with the explantation technique previ-
ously described (5). The studies were carried out using 
cells in Passages 5 to 8. Bulk preparation of cells for the 
experiments was made in BM supplemented with HS and 
FBS at concentrations of 10% (vol/vol) each. This 
medium was designated S-BM and has been found to 
induce a rapid proliferation of hASMC in vitro (5).

Growth arrest of sparse cells was achieved either by 
adding heparin (10 g/liter) to S-BM or by replacing the 
sera with 1% (wt/vol) BSA. These media were 
designated HEP-BM and BSA-BM, respectively, and their 
effects on hASMC proliferation have been described (5).

Cells were harvested by trypsinization and passed as 
previously described (5). The counting of cells was done 
in an AI Cell Counter 134 (AnalyS Instrument, Solna, 
Sweden).

**Immunofluorescence studies of cells in culture.** In all 
experiments the cells were seeded in S-BM on gelatin-
coated glass cover slips in 10-cm² petri dishes. The 
medium was changed after 16 to 18 h. Thereafter, the 
medium was changed every 3rd d.

Proliferating cells were studied in sparse cultures 
seded at 2 × 10⁶ cells/cm² and grown for 4 d in S-BM 
before preparation for immunofluorescence of contracti-
tile proteins. Growth-arrested cells were also studied in 
sparse cultures. They were seeded at 10³ cells/cm², 
grown for 4 d in S-BM and for another 6 d in BSA-BM or 
HEP-BM before immunofluorescence. Restimulated 
cells were prepared in the same way as growth-arrested 
cells, except that growth-arrest in BSA-BM or HEP-BM 
was followed by incubation in S-BM, and cover slips were 
taken for immunofluorescence of contractile proteins 
both 3 and 6 d later.

Expression of the PC nuclear antigen and SMC-specific 
contractile proteins in sparse hASMC cultures was 
studied in a time-course experiment. hASMC were seeded 
on gelatin-coated glass cover slips at 10⁶ cells/cm² as 
before and grown in S-BM for 2 d. Growth arrest was then 
induced by culture in BSA-BM for 7 d and, finally, the 
cells were restimulated to grow in S-BM for another 7 d. 
During this incubation, triplicate cover slips were taken