TR3 nuclear orphan receptor in cardiovascular disease
Arkenbout, E.K.

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TR3 orphan receptor is expressed in vascular endothelial cells and mediates cell cycle arrest

E. Karin Arkenbout, Maaike van Bragt, Eric Eldering*, Chris van Bree**, Jos M. Grimbergen#, Paul H.A. Quax#, Hans Pannekoek, Carlie J.M. de Vries

From the Departments of Biochemistry, Experimental Immunology and Radiotherapy, Academic Medical Center, University of Amsterdam, Amsterdam
#Gaubius Laboratory TNO-PG, Leiden, The Netherlands
Abstract

Objective

Endothelial cells play a pivotal role in vascular homeostasis. In this study, we investigated the function of the Nerve growth factor-induced protein-B (NGFI-B) subfamily of nuclear receptors comprising TR3 orphan receptor (TR3), mitogen-induced nuclear orphan receptor (MINOR) and nuclear orphan receptor of T-cells (NOT) in endothelial cells.

Methods and Results

The mRNA expression of TR3, MINOR and NOT in atherosclerotic lesions was assessed in human vascular specimens. Each of these factors is expressed in smooth muscle cells as described before, and in subsets of endothelial cells, implicating that they may affect endothelial cell function. Adenoviral overexpression of TR3 in cultured endothelial cells resulted in decreased \[^{3}H\]-thymidine incorporation, whereas a dominant-negative TR3 variant that inhibits the activity of endogenous TR3-like factors enhanced DNA synthesis. TR3 interfered with progression of the cell cycle by upregulating p27\(^{kip1}\) and downregulating Cyclin A, whereas expression levels of a number of other cellcycle-associated proteins remained unchanged.

Conclusions

These findings demonstrate that TR3 is a modulator of endothelial cell proliferation and arrests endothelial cells in the G1 phase of the cell cycle by influencing cell-cycle protein levels. We hypothesize involvement of TR3 in maintenance of the integrity of vascular endothelium.

Condensed abstract

TR3 orphan receptor (TR3)-like transcription factors are expressed in endothelial cells in vascular (atherosclerotic) tissue and in capillary endothelial cells. Adenoviral overexpression of TR3 in cultured endothelial cells reduced DNA synthesis and provoked G1-arrest of the cell cycle, which correlated with enhanced p27\(^{kip1}\) and reduced Cyclin A expression.
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Introduction

Under physiological conditions vascular endothelium serves as a physical barrier between the blood compartment and the vessel wall and remains in a quiescent, non-proliferative state.\(^1\) Endothelial cell proliferation is, however, induced in pathological situations e.g. by balloon angioplasty-mediated disruption of the monolayer. The exact molecular mechanism regulating endothelial cell proliferation under pathophysiological conditions are poorly understood. Endothelial cell-cycle progression requires activation of distinct cyclin-dependent kinases (Cdk) through their association with regulatory subunits called cyclins, during different phases of the cell cycle.\(^2\) The Cdk inhibitors p\(21^{Cip1}\), p\(27^{Kip1}\), and p\(15^{INK}\) negatively regulate this process by inhibiting cyclin/Cdk activity.\(^3\) In ECs and a variety of other cells, the Cdk inhibitor p\(27^{Kip1}\) induces G\(1\) arrest of the cell cycle and inhibits growth and migration.\(^4\) The Nerve growth factor-induced protein-B (NGFI-B) subfamily of nuclear receptors (NR4A) belongs to the steroid/thyroid hormone superfamily of transcription factors and comprises TR3 orphan receptor (TR3), mitogen-induced nuclear orphan receptor (MINOR) and nuclear orphan receptor of T-cells (NOT).\(^5\) Like other members of the nuclear receptor superfamily, the NGFI-B-like factors contain a central DNA-binding domain, comprising two Zn-fingers, that recognizes response elements in the promoter of specific target genes. The amino-terminal domain mediates transactivation and the carboxy-terminal domain is involved in (hetero)dimerization and ligand binding. At present, the ligands for TR3, MINOR and NOT are unknown, qualifying these transcription factors as orphan receptors.\(^6\) Several lines of evidence indicate that TR3 and MINOR are involved in T-cell apoptosis. Antisense oligo-nucleotides directed against TR3 prevent apoptosis in cultured T-cells and prostate and lung carcinoma cells.\(^7\) Furthermore, it has been shown that overexpression of TR3 or MINOR in developing T-cells of transgenic mice results in massive apoptosis of thymocytes and reduced levels of peripheral T-cells, whereas a dominant-negative variant of TR3 inhibits T-cell apoptosis.\(^8\) Recently, it has been shown that in response to apoptotic stimuli TR3 can translocate from the nucleus to mitochondria to promote cytochrome c release and apoptosis. This pro-apoptotic effect of TR3 was shown to be independent of its transactivation activity.\(^9\)

We have recently shown that TR3 inhibits serum-stimulated proliferation of vascular smooth muscle cells (SMCs). Moreover, overexpression of TR3 specifically in arterial SMCs of transgenic mice resulted in reduced intima formation in the carotid artery ligation-model.\(^10\) Downregulation of p\(27^{Kip1}\) is crucial for cell-cycle progression and we showed that TR3 controls progression of the cell cycle in SMCs through the regulation of this important Cdk inhibitor.\(^11\)
In the current study, we show expression of the NGFI-B family members in ECs in human vascular tissue. We demonstrate that TR3 regulates \( p27^{kip} \) and Cyclin A protein levels in cultured ECs, consistent with inhibition of DNA synthesis and arrest of the cell cycle at \( G_1 \). We discuss the physiological relevance of TR3-like factors in protection against excessive vascular endothelial cell proliferation.

**Methods**

*Immunohistochemistry and radioactive in situ hybridization*

Human tissue samples were obtained with informed consent from organ donors, according to protocols approved by the Medical Ethical Committee of the Academic Medical Center, Amsterdam. The specimens were paraffin-embedded, sectioned and mounted on Superfrost Plus glass slides (Emergo, Belgium). Vascular specimens were characterized by immunohistochemistry to establish the stage of disease according to the American Heart Association classification. ECs in the human vascular specimens were identified by Ulex europaeus lectin (1:50 dilution), which was detected with a rabbit anti Ulex-lectin antibody conjugated with horseradish peroxidase at a 1:50 dilution (DAKO, Denmark). Radioactive in situ hybridization assays were performed as described. The following riboprobes were synthesized for in situ hybridization: TR3, Genbank No. L13740, bp 1221-1905; MINOR, Genbank No. U12767, bp 1435-2172; NOT, Genbank No. X75918, bp 119-1003. Matching sense riboprobes were assayed for each gene and were shown to give neither background nor an aspecific signal. The sections were exposed for 4 to 8 weeks.

*HUVEC isolation and cell culture*

Human umbilical vein ECs (HUVEC) were isolated as described and cultured on fibronectin-coated tissue culture plates (NUNC, Napierville, IL) in 'growth medium' containing Medium-199 (GIBCO-BRL, Paisley, Scotland), supplemented with 20% (v/v) fetal bovine serum, 2mM L-Glutamin, 50 mg/ml heparin (Sigma, St. Louis, MO), 12.5 mg/ml Endothelial Cell Growth Supplement (ECGS) (Sigma) and 100 U/ml penicillin/streptomycin (GIBCO-BRL). Cells at passage level 1-2 were applied and plated on fibronectin-coated 6-wells plates (NUNC) in growth medium.

*Recombinant adenoviruses*

Replication-defective adenoviruses expressing cDNAs under control of the CMV-promoter were purified by Cesium Chloride-gradient centrifugation and viral titers were determined by standard plaque assays. Full-length TR3 cDNA bp 1-2375 (Genbank X97226) was inserted in the TR3-virus, whereas mock virus did not contain cDNA sequence. The ATA-
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virus lacked bp 178-690 from the full-length cDNA and consequently encodes a TR3-variant without the transactivation domain. ATA exhibits normal DNA-binding without mediating transcriptional activation, and acts as a dominant-negative inhibitor for all three subfamily members TR3, MINOR and NOT.

\[^{3}H\]Thymidine incorporation

HUVEC were seeded in 24-well plates and reached 70-80\% confluency after 24 h. Cells were infected for 2 h with mock-, ATA- or TR3 adenovirus at 3 x 10^5 pfu/mL in modified growth medium containing 5 \(\mu\)g/mL ECGS (instead of 12.5 \(\mu\)g/mL) and were allowed to recover in full growth medium for 2 h. Subsequently, the cells were maintained for 60 h in modified growth medium containing 5 \(\mu\)g/mL ECGS. The HUVEC were stimulated with growth medium and after 8 h 0.5 \(\mu\)Ci/mL [methyl-\(^{3}H\)]-thymidine (Amersham, Buckinghamshire, UK) was added for 18 h. Incorporated radioactivity was determined as described. The experiments were performed in triplicate and repeated three times.

Western Blotting

SDS-polyacrylamide gel electrophoresis was performed with cell lysates and proteins were transferred to nitrocellulose-Protran (Schleicher and Schuell, Germany). TR3 was detected by Western blotting with an antiserum directed against Nur77 (M-210, 1:1000), Jab1 was detected with antiserum sc-6271 (1:200) (Santa Cruz Biotechnology, CA), pro-Caspase 3 protein was assayed with an antiserum from BD Biosciences (CA) (1:1500), whereas cleaved Caspase 3 was shown with an antiserum (D175; 1:1000) from Cell Signaling (MA). Monoclonal antibodies were used to detect Cdk1/Cdc2 (1:2500), Cdk2 (1:2500), Cyclin A (1:250), Cyclin B (1:1000), Cyclin D, (1:1000), PCNA (1:5000), p21WAF1/Cip1 (1:500), p27Kip1 (1:1000) (BD Biosciences, CA), and \(\alpha\)-tubulin (1:2500) (Cedar Lane, Canada). Proteins were visualized by ECL detection (Amersham, Germany) and the Lumi-Imager (Boehringer Mannheim, Germany).

Flowcytometric analysis of cell cycle distribution

Cells were plated in 6-well plates and infected as described above to assess \(^{3}H\)-thymidine incorporation. After recovery of the cells for 2 h in growth medium, the cells were incubated for 60 h in growth medium with low (5 \(\mu\)g/mL) ECGS. Subsequently, the HUVEC were split 1:2 and placed overnight in growth medium. Bromodeoxyuridine (BrdU) was administered from a 100x stock solution to a final concentration of 10 mM. After 4 h the cells were harvested, fixed in 70\% ethanol in phosphate-buffered saline (PBS), and stored
at -20°C. Ethanol-fixed cells were centrifuged (1 min, 2200 rpm), resuspended in 1 mL pepsin solution (0.4 mg/mL 0.1N HCl), and incubated for 30 min at room temperature. Subsequently, the DNA was degraded by a 30-min incubation at 37°C in 1 mL 2N HCl. After washing with PBTr (PBS, Tween-20 0.05% v/v, bovine serum albumin [Sigma] 20 mg/mL), the cells were resuspended in 0.1 mL rat-anti- BrdU (Harlan Sera-Laboratory Ltd., Loughborough, UK, diluted 1:100 in PBTb) and incubated for 30 min at room temperature. After washing with PBTrg (PBS, Tween-20 0.05% v/v, normal goat serum [Dako, Glostrup, Denmark] 1% v/v), the cells were resuspended with 0.1 mL fluorescein isothiocyanate (FITC)-conjugated goat-anti-rat IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA, diluted 1:100 in PBTg) and incubated in the dark for 30 min at room temperature. Propidium iodine and ethanol were added to an end-concentration of 1 mg/mL and 30% (v/v) respectively. Samples were syringed through a 21-gauge needle to reduce cell aggregation before flowcytometry (FACScan cytometer, Becton Dickinson, San Jose, CA).

**Real-time RT-PCR**

Cells were plated in 6-well plates and infected and cultured as described above for the flowcytometric analyses. Total RNA was extracted and reverse transcription of 1 µg of total RNA was performed with 0.5 µg (dT)$_{18}$ primer using SuperScript II (GIBCO-BRL). Real-time RT-PCR was performed using the FastStart DNA Master SYBR Green I kit (Roche, Mannheim, Germany) in the LightCycler System (Roche). Primers were TR3, (forward) 5'-GTTCTCTGGAGTGTCATCCGCAAG -3' and (reverse) 5'-GCAGGGACCTTTGAGAAGGCCA-3'; MINOR, (forward) 5'-CCATTACAACAGGAACCTTC-3' and (reverse) 5'-GAGATCAGTAAATCCCGGAA-3'; NOT, (forward) 5'-TATlï;CAGGTTCCAGGCGAA-3' and (reverse) 5'-GCTAATCGAAGGACAAACAG-3'. As a control for equal amounts of first-strand cDNA in the different samples, we determined HPRT mRNA levels; (forward) 5'-TAATTATGGAACAGGACTGAACG-5', and (reverse) 5'-CACAATCAAGACATTTCTTCCAG-3'.

**Results**

**TR3, MINOR and NOT are expressed in ECs in vivo**

We have previously shown that TR3, MINOR and NOT are expressed in intimal SMCs of human atherosclerotic lesions, whereas no expression was observed in normal, medial SMCs. To determine whether these genes are expressed in arterial ECs, we performed radioactive in situ hybridizations with probes specific for TR3, MINOR or NOT. We analyzed the expression of TR3-like factors in 19 different vascular specimen derived from individuals.
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ranging in age from 17 to 65 yrs. The complexity of the lesions varied from apparently normal, Type I to Type V according to the classification of the American Heart Association. In apparently healthy endothelium we observed patchy, non-continuous expression of TR3, MINOR and NOT (Figure 1a-c, respectively). Furthermore, expression of TR3-like factors was present in the vasa vasorum of normal and atherosclerotic vascular tissue (Figure 1d-f). TR3-like factors are prominently expressed in microvascular ECs as well as in some cells surrounding the capillaries, which are probably adventitial fibroblasts (indicated by arrows). As a control, in situ hybridizations were performed with sense riboprobes (Figure 1 g-i). Next we show expression of TR3, MINOR and NOT in Type II lesions (Figure 2). An overview of the specimens after hematoxylin/eosin (HE)-staining is shown (Figure 2 a, d and g) and the corresponding endothelial cell-specific staining illustrates the presence of an intact endothelial layer (Figure 2b, e and h). In situ hybridizations were performed on consecutive sections and revealed mRNA expression of TR3, MINOR or NOT in all ECs as well as in some underlying intimal SMCs (Figure 2 c, f and i). These experiments revealed that ECs overlying atherosclerotic lesions express mRNA encoding each of these transcription factors.

Adenoviral infection of HUVEC to overexpress TR3 and the dominant-negative variant of TR3 (ADA)

The mRNA expression studies revealed relatively high expression of TR3 in ECs in atherosclerotic lesions, which prompted us to assess the functional activity of TR3 in cultured human umbilical cord ECs (HUVEC). We applied adenoviruses expressing either TR3 or a dominant-negative variant of TR3, denoted ADA. This dominant-negative variant lacks the amino-terminal transactivation domain and inhibits endogenous transcriptional activity of all three family members, which is of importance because functional redundancy has been described for members of this subfamily.

We first assayed the expression of TR3, MINOR and NOT in HUVEC under the conditions used in our experiments. Confluent, quiescent HUVEC cultures were exposed to complete growth medium and the expression of TR3, MINOR and NOT was monitored by real-time RT-PCR (Figure 3A). All three nuclear receptors are transiently induced in serum-stimulated HUVEC, with optimal expression 2-4 h after stimulation.

Since TR3 has been associated with apoptosis, HUVEC were infected with mock-, ADA- or TR3-adenovirus and the extent of Caspase 3 activation was determined by Western blotting using an antiseraum specific for cleaved Caspase 3 (19 kD). Clearly, no activation of Caspase 3 was observed in the infected cells, whereas treatment with the apoptotic agent staurosporin
Figure 1. Expression of TR3, MINOR and NOT mRNA in normal vascular endothelium. (a) TR3, (b) MINOR and (c) NOT expression was determined by radioactive in situ hybridization in which positive signal results in black dots on a purple nuclear counterstain (hematoxilin). Each of these genes showed a patchy expression in ECs of apparently healthy vessels. In addition, (d) TR3, (e) MINOR and (f) NOT mRNA was expressed in adventitial microvessels. As a control for probe specificity, hybridizations were performed with sense riboprobes (g-i) The red lines indicate internal elastic laminae (a-c). The arrows in d-e indicate positive cells in the adventitia, probably myofibroblasts. Original magnifications 200x (a-c), 630x (d-i).

during 2 h resulted in generation of a substantial amount of active Caspase 3 (Fig. 3). Inactive pro-Caspase 3 (36 kD) was present in all cells, with enhanced expression in ΔTA-infected cells. Pro-Caspase 3 was relatively low in staurosponin-treated cells, which correlated with less total protein loaded in this lane as illustrated by a-tubulin expression levels. Given that only the presence of cleaved Caspase 3 is indicative for the induction of apoptosis, we concluded that overexpression of TR3 or ΔTA does not affect programmed cell death in ECs.

To determine whether TR3 is involved in cellular proliferation, we performed [3H]-thymidine incorporation experiments. Overexpression of TR3 resulted in a 2.6 fold lower [3H]-thymidine incorporation and, consistent with these data, ΔTA-infected HUVEC showed a 1.5 fold increase in DNA-synthesis (*P<0.001) (Figure 4A). Moreover, TR3 blocks the cell
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cycle in ECs in the G1-phase as shown by FACS analysis of propidium-iodide stained cells (Figure 4B). The number of cells in the S-phase was determined by BrdU-staining followed by FACS analysis and was shown to be 2.1-fold lower in the TR3-infected cells compared to the mock-infected cells (13.1% and 28.1% of the counted cells, respectively). To delineate the mechanism of this cell cycle arrest, we determined the expression level of cell-cycle proteins by Western blotting, followed by quantitative Luminometry (Figure 5). No changes were observed in protein expression levels of Cdk1, Cdk2, Cyclin B, Cyclin D3, PCNA and p21\(^{CIP1}\) (Figure 5A). The expression of the Cdk inhibitor p21\(^{CIP1}\) was upregulated 2-fold by TR3 and downregulated 2.2-fold by ATA, whereas Cyclin A was regulated inversely; 1.9-

![HE](image1)

**HE**

![Endothelial cells](image2)

**Endothelial cells**

![In situ hybridization](image3)

**In situ hybridization**

Figure 2. TR3, MINOR and NOT mRNA expression in human atherosclerotic lesions. Serial sections of three different human lesions were investigated by (a, d and g) hematoxilin/eosin (HE)-staining; an overview of the vessel is shown. (b, e and h) Immunohistochemistry with Ulex-staining (see Materials and Methods) to detect ECs that are shown at a larger magnification. (c, f and i) Radioactive in situ hybridization; homogeneous expression of (c) TR3, (f) MINOR and (i) NOT mRNA in ECs on atherosclerotic lesions. Arrows in f and i indicate positive SMCs. M=media, N=neointima, A=adventitia. Original magnifications 25x (a, d, g), 400x (b, e, h), 630x (c, f, i).
fold downregulation by TR3 and 2.6-fold upregulation by ΔTA (Figure 5 B-C). It has been described that p27Kip1 is a substrate of the ubiquitin/proteasome system and that Jab1 controls the activity of p27Kip1 by facilitating its degradation. However, Jab1 protein levels were not affected by TR3 or ΔTA (Figure 5A).

Figure 3. TR3, MINOR and NOT are expressed in HUVEC and are not involved in apoptosis. (A) Real-time RT-PCR analysis of TR3, MINOR and NOT expression in HUVEC demonstrated transient expression in serum-stimulated HUVEC. (B) HUVEC were infected with mock-, ΔTA- or TR3-adenovirus or were treated for 2 h with staurosporin. Cleaved Caspase 3 (19 kD) was detected with an antibody specific for the activated form of Caspase 3. Only in staurosporin-treated HUVEC activated Caspase 3 was observed. Uncleaved, inactive Caspase 3 protein (36 kD) was detected in all cell lysates. The expression of α-tubulin was shown as a control for equal protein loading. TR3 does not induce apoptosis in HUVEC.

Figure 4. Adenovirus-mediated overexpression of full-length TR3 or ΔTA in cultured HUVEC to study DNA synthesis and cell cycle progression. (A) HUVEC were infected with mock-, ΔTA- or TR3-adenovirus and DNA synthesis was investigated by 3H-thymidine incorporation; ΔTA enhances and TR3 inhibits DNA synthesis (* = P<0.001). (B) Bivariate flow cytometry with propidium iodide (PI) fluorescence indicating the amount of DNA on the x-axis and FITC-fluorescence indicating BrdU incorporation on the y-axis. Populations in G1-, S- and G2 phase are indicated. TR3-infected cells exhibited G1 arrest compared to mock-infected cells.
Figure 5. Adenovirus-mediated overexpression of ΔTA and TR3 in cultured HUVEC to study cell-cycle protein expression. (A) HUVEC were infected with mock-, ΔTA- or TR3-adenovirus and cell lysates were assayed for cell cycle-associated protein expression by Western blotting. (B) The expression of p27^Kip1 and Cyclin A was affected by ΔTA and TR3, whereas the expression level of a number of other cell-cycle proteins and Jab1 was not changed (A). The expression of α-tubulin was assayed as a control for equal protein loading. (C) Luminometry allowed quantitative analysis of the expression levels of p27^Kip1 and Cyclin A.

Discussion

ECs constitute a monolayer in the vessel wall facing the lumen and display a strategic function in regulation of many (patho) physiological processes such as the control of blood coagulation, vasomotor tone, ischemic and reperfusion injuries, and atherosclerosis. In pathological situations, such as the initiation and progression of atherosclerosis, ECs become activated and are involved in excessive extravasation of monocytes into the vessel wall. In response to local insults for example due to transluminal angioplasty the endothelium is disrupted resulting in a proliferative response of these cells.

In the current study, we demonstrated that the nuclear orphan receptor TR3, and potentially also its subfamily members NOT and MINOR, induce cell-cycle arrest of ECs. TR3, MINOR and NOT were shown to be expressed in ECs in the (atherosclerotic) vessel wall. The
majority of the ECs overlying atherosclerotic plaques in large arteries express TR3, MINOR and NOT mRNA. In the apparently healthy part of vessels with relatively early, eccentric lesions we observed patchy expression of TR3-like factors in the EC layer. TR3-like factors have been implicated in apoptosis of T-cells, prostate tumour and lung tumour cells. We have shown that TR3 overexpression in ECs or full inhibition of the transcriptional activity of endogenous subfamily members by ATA in these cells does not affect the activation of Caspase 3. Furthermore, we performed a Multiplex ligation-dependent probe amplification, which allowed simultaneous assessment of mRNA expression levels of 24 apoptosis-related genes and showed that both TR3- and ATA-overexpressing HUVEC exhibit no significant differences in the expression of each of these genes (unpublished data). Based on these data, we concluded that TR3 does not provoke apoptosis in ECs.

Here, we demonstrate inhibition of endothelial cell proliferation in response to TR3 overexpression, which may be explained by Cyclin A downregulation and enhanced expression of the Cdk inhibitor p27kip1, resulting in cell cycle arrest at G1 as shown by FACS analysis. Analogous to our data Chen et al have shown that p27kip1 induction and Cyclin A downregulation are crucial for cell-cell contact mediated inhibition of endothelial cell proliferation. Furthermore, Hirano et al have concluded that cell-cell contact mediated growth inhibition in vascular ECs involves to some extent transcriptional upregulation of the p27kip1 gene. It is thus conceivable that TR3 modulates p27kip1 expression at the transcriptional level, even though no consensus NGBF1-B response element (NBRE) or NurRE have been identified in the region 5 kb upstream of the p27kip1 gene (unpublished data). An alternative mechanism may be that Jab1-mediated degradation of p27kip1 is less rapid in the presence of TR3, however, we did not find any change in Jab1 expression levels in ATA- or TR3-overexpressing HUVEC, implying that this is probably not the mechanism by which TR3 influences p27kip1 protein expression levels. Tanner et al have demonstrated that in human atherosclerotic lesions p27kip1 expression appears to correlate inversely with vascular cell proliferation and they proposed that p27kip1 sets the balance between proliferating and nonproliferating cells in the vessel wall. Moreover, p27kip1 knock-out mice crossed with ApoE knock-out mice exhibit a profound enhancement of atherosclerosis compared to ApoE knock-out mice when fed a Western-type diet, indicating that p27kip1 protects against diet-induced atherosclerosis. However, it is difficult to assess the effect of the lack of p27kip1 expression on endothelial cell (dys)function and its relative contribution to lesion formation in those in vivo experiments.

In adventitial microvessels we observed relatively high mRNA levels of TR3, MINOR and NOT. These data are in good correlation with the observation of abundant and uniform
expression of \( p27^{Ki1} \) in adventitial microvessels.\(^{27}\) In the same study \( p27^{Ki1} \) was shown to be expressed in a patchy manner in luminal ECs of large arteries, which correlates with the vascular expression pattern that we describe for TR3.

Finally, endothelial cell proliferation is crucial for angiogenesis and, consequently, anti-angiogenic therapy by inducing growth arrest of endothelial cell has been shown to be effective in the treatment of cancer.\(^{29}\) Accordingly, it has been shown that overexpression of \( p27^{Ki1} \) inhibits endothelial cell proliferation, resulting in reduced angiogenesis\(^{5}\) and delayed tumor progression.\(^{30}\) Therefore, TR3 may be considered as a potential target to inhibit angiogenesis. However, in vivo experiments are necessary to provide evidence for such an effect of TR3.

In summary, we show that TR3, MINOR and NOT are expressed in (activated) ECs and that TR3 inhibits proliferation of these cells. Endothelial cell growth inhibition may be undesirable in situations where the endothelium is denuded such as after angioplasty. At the initiation of atherosclerosis, however, the endothelial cell layer is not disrupted. In this respect it is important to mention that TR3 has also been shown to cause cell-cycle arrest in the dopaminergic NM9D cell line, which simultaneously results in induction of differentiation of these cells.\(^{31}\) We envision that expression of TR3 in ECs results, by an analogous mechanism, in preservation of the normal endothelial cell characteristics, thereby influencing the vessel wall towards a reduction of its susceptibility to progression of atherosclerosis.

Recently, Gruber et al. showed similar data on TR3 (denoted as Nur77/NAK1) expression in atherosclerotic vessels and showed that TR3 is expressed in HUVEC in response to tumor necrosis factor-\( \alpha \), interleukin-1 and lipopolysaccharide stimulation.\(^{25}\)

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Chapter 4

References


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