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[16] Trinuclear {Cp*M^{III}} complexes (M = Rh, Ir) with bridging amino carboxylates[5] or nucleobases[3b, 6] were reported by Beck, Fish, and Carmona. Some of these "bioorganometallic" compounds can act as a host for aromatic amino acids[6] or show interesting catalytic behavior.[5]

[17] Crystal structure analysis: General: Siemens CCD area detector, $Mo_{K\alpha}$ radiation, $\lambda = 0.71073$ Å, semiempirical absorption correction with SADABS. The structure was solved with direct methods (SHELXS-97, Sheldrick, 1990). Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-127378 (**2**) and CCDC-127377 (**4**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk). Crystal structure analysis of $\mathbf{2}\cdot 0.5\,CHCl_3$: crystal size $0.02 \times 0.01 \times 0.01$ mm. The crystal was mounted in perfluoropolyether oil, $T = 173$ K, yellow prism, hexagonal, space group $R\bar{3}$, $a = b = 19.6202(6)$, $c = 21.9013(9)$ Å, $V = 7301.4(4)$ Å^3, $Z = 6$, $\rho_{calcd} = 1.926$ Mg m^{-3}, $\mu = 8.313$ mm^{-1}. Data collection: 2θ from 3.04 to 59.02, $-25 \leq h \leq 25$, $-24 \leq k \leq 24$, $-28 \leq l \leq 24$, 14799 reflections collected, 3515 independent reflections, 2590 observed reflections ($F > 4\sigma(F)$), max./min. transmission 0.9215/0.8514, $R_1 = 0.0471$, $wR_2 = 0.1038$ ($F > 4\sigma(F)$), GOF(F^2) = 1.047, residual electron density 5.080/-2.994 e Å^{-3}, the weighting scheme is $w^{-1} = \sigma^2 F_o^2 + (0.0477P)^2 + 221.7031P$ with $P = (F_o^2 + 2F_c^2)/3$. A riding model was employed for the hydrogen atoms. There is a high electron density near the Ir atom (0.78 Å, 5.08 e Å^{-3}). The solvent molecule is only partially occupied (GOF = 0.1666). Crystal structure analysis of $\mathbf{4}\cdot$THF: crystal size $0.20 \times 0.10 \times 0.10$ mm. The crystal was mounted in perfluoropolyether oil, $T = 183$ K, red prism, monoclinic, space group $C2/c$, $a = 35.694(2)$, $b = 14.6090(7)$, $c = 22.456(1)$ Å, $\beta = 123.527(1)^\circ$, $V = 9761.3(8)$ Å^3, $Z = 8$, $\rho_{calcd} = 1.550$ Mg m^{-3}, $\mu = 0.970$ mm^{-1}. Data collection: 2θ from 3.10 to 58.60, $-44 \leq h \leq 44$, $-18 \leq k \leq 18$, $-27 \leq l \leq 28$, 28663 reflections collected, 9848 independent reflections, 6759 observed reflections ($F > 4\sigma(F)$), max./min. transmission 0.9092/0.8296, $R_1 = 0.0356$, $wR_2 = 0.0797$ ($F > 4\sigma(F)$), GOF(F^2) = 0.943, residual electron density 1.013/-0.786 e Å^{-3}, weighting scheme $w^{-1} = \sigma^2 F_o^2 + (0.0488\ P)^2 + 0.0000P$ with $P = (F_o^2 + 2F_c^2)/3$. For the hydrogen atoms a riding model was employed. The disordered solvent molecule was refined isotropically and no hydrogens were added.

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[19] The formation of trinuclear rhodium complexes with bridging 9-methylhypoxanthine ligands from monomeric or dinuclear complexes was shown to be pH dependent.[6b]

Inhibition of Angiogenesis In Vivo by *ets-1* Antisense Oligonucleotides—Inhibition of Ets-1 Transcription Factor Expression by the Antibiotic Fumagillin**

Nicolas Wernert,* Antje Stanjek, Serafim Kiriakidis, Anja Hügel, Hem Chandra Jha, Ralph Mazitschek, and Athanassios Giannis*

Dedicated to Professor Konrad Sandhoff on the occasion of his 60th birthday

The formation of new blood capillaries from preexisting ones (angiogenesis or neovascularization; Scheme 1) is a fundamental aspect of many physiological and pathological processes[1] such as reproduction, embryonic development, wound healing, chronic inflammation, and malignant growth. Folkman's view of the early 1970s[2] that an appropriate blood supply is necessary for tumor growth has been confirmed meanwhile. Furthermore, subsequent investigations have shown that not only tumor growth but also tumor metastasis is dependent on angiogenesis.[3] For these reasons anti-angiogenesis became an attractive strategy for the treatment of neoplastic diseases.[4–7] Angiogenesis inhibitors are likewise useful for the treatment of other frequent angiogenesis-dependent diseases such as diabetic retinopathy[8] and rheumatoid arthritis.[9]

The mechanisms of angiogenesis have been intensively investigated during the last years, and several endogenous regulators have been identified.[10] Vascular endothelial

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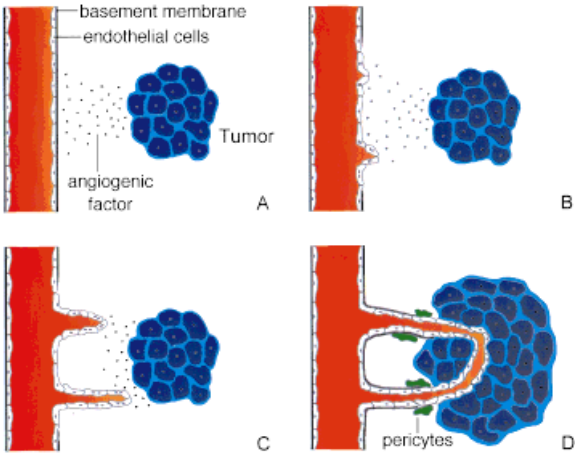

Scheme 1. The complex process of angiogenesis comprises the following steps: A) Activation of the endothelial cell by growth factors. B) Degradation of the basement membrane of the blood vessels by proteinases (collagenases, plasminogen activator). C) Migration and proliferation of endothelial cells towards the angiogenic stimulus. Matrix metalloproteinases are recruited for extracellular matrix remodeling during this step. D) Formation of a new basement membrane around the immature blood vessels and merging of the ends of two outgrowing blood vessels (anastomosis). In general, tumor vascularization also stimulates tumor growth. Modified according to ref. [5, 33].

growth factor (VEGF), a protein with several isoforms, is considered to be the most important stimulator of angiogenesis.[11] The different VEGF isoforms bind to two tyrosine kinase receptors, VEGF-R1 (*flt-1*) and VEGF-R2 (*flk-1*), both of which are almost exclusively present on the plasma membrane of endothelial cells.[12] Activation of VEGF receptors leads by the Ras-Raf-MAP-kinase pathway to the expression[11] of proteinases (serine proteinases, cysteine proteinases, and matrix metalloproteinases) and of specific integrins on endothelial cell surfaces, and finally initiates the proliferation and the migration of these cells towards the angiogenic stimulus. The Ets-1 transcription factor[13, 14] plays an important role for the regulation of neovascularization. Some of the genes which encode the above-mentioned proteinases contain within their promotor sequences Ets binding sites with the central core motif GGAA/T. Ets-1 binds to these regulatory control regions through a helix–turn–helix motif and enhances the expression of these proteinases.

We were able to show that the expression of Ets-1 proteins is stimulated during tumor-induced angiogenesis.[15, 16] Recently it has also been reported that stimulation of human endothelial cells with VEGF leads to an induction of Ets-1 expression.[17] Interestingly enough, the Ets-1 protein is also involved as a positive regulatory factor in the transcriptional control of the gene encoding VEGF-R1 (*flt-1*).[18] However, the importance of Ets-1 for in vivo angiogenesis has not been demonstrated yet.

Here we report for the first time that *ets-1* antisense oligodeoxynucleotides (ODN) effectively reduce the formation of new blood vessels on the chick chorioallantois membrane (CAM-assay[19]). Additionally, we show that the potent angiogenesis inhibitor fumagillin (**3**) strongly inhibits Ets-1 expression.

To block Ets-1 expression in the CAM assay we used the phosphorothioate antisense ODN 5′-AGATC-GACGGCCGCCTTCAT-3′ (**1**), which inhibits Ets-1 expression in cultured endothelial cells.[17] This 20-mer is complementary to the AUG inition codon and a short "downstream" sequence of the *c-ets-1* mRNA. We used the sense phosphorothioate 5′-ATGAAGGCGGCCGTCGATCT-3′ (**2**) as a negative control. We directly applied three different concentrations of either antisense or sense ODNs (2.5, 5, and 10 μg per egg; the ODNs were dissolved in 5 μL of 150 mM NaCl and 5 μL of transfection solution,[20] respectively) on the chicken chorioallantois membrane on day 5 of development and evaluated the results on day 7. A drastic reduction of angiogenesis was observed after application of 5 μg of antisense ODN **1** (seven embryos compared to eight embryos of the sense control group). Both the number and the diameter of blood vessels were significantly reduced (Figure 1 c–g). No effect was detected in either the antisense group (two embryos) or in the sense group (three embryos) after application of 2.5 μg of ODN. All six embryos in the antisense group and five of six embryos in the control group had died by day 7 following the application of 10 μg of sense or antisense ODN. We observed no inhibition of angiogenesis when using the transfection solution alone (Figure 1 h).

Since the biosynthesis rate of the Ets-1 transcription factor is greatly enhanced during angiogenesis under both physio-

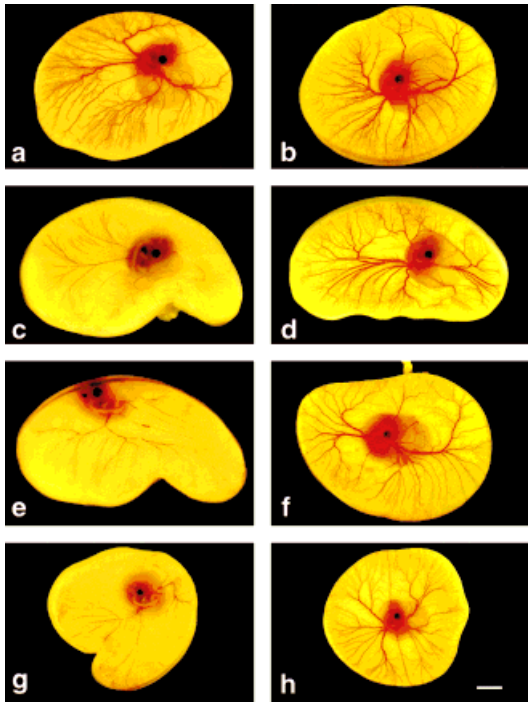

Figure 1. Inhibition of blood vessel development on the chick chorioallantois membrane by *ets-1* antisense ODN **1**. No angiostatic effect was detected after application of 2.5 μg of antisense ODN in 10 μL of transfection solution (a) or of 2.5 μg of sense ODN in 10 μL of transfection solution (b). In contrast, a strong inhibition of angiogenesis was observed after application of 5 μg of antisense ODN in 10 μL of transfection solution (c, e, g; examples of different embryos). Compared to the sense control group (d, f; in each case 5 μg of sense ODN in 10 μL of transfection solution), both the number and the diameter of blood vessels were significantly reduced. No angiostatic effect was observed after application of the transfection solution alone (h, bar = 1 cm). The oligodeoxynucleotides were purchased from Applied Biosystems, Weiterstadt (Germany), and the transfection solution was purchased from Euromedex, Strasbourg (France).

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logical and pathological conditions, a participation of Ets-1 in in vivo blood vessel formation has strongly been suggested. When angiogensis is finished, Ets-1 expression is downregulated again.[21] The results obtained in the present study prove for the first time the in vivo role of the Ets-1 transcription factor in neovascularization. This is further supported by investigations in cultured endothelial cells[17] in which inhibition of Ets-1 expression by the antisense ODN **1** not only diminishes cell proliferation and migration but also the production of urokinase-type plasminogen activator and collagenase I. Both enzymes are necessary for extracellular matrix remodeling during angiogenesis.[5, 22, 23]

In view of these findings the Ets-1 transcription factor represents a promising target in experimental cancer therapy.[13] In addition to antisense strategies, low molecular weight compounds which inhibit Ets-1 expression seem particularly useful. We assumed that the fungal metabolite fumagillin (**3**),[24] which strongly inhibits both endothelial cell proliferation in vitro and angiogenesis in vivo, could exert its effects by modulating Ets-1 expression. To investigate this hypothesis, we stimulated human umbilical vein endothelial cells (HUVEC) with VEGF in the absence or in the presence of fumagillin and subsequently investigated Ets-1 expression by Western blotting. Four hours after application of VEGF (150 ng) we observed a significant increase of both the p51 and the p39 Ets-1 proteins.[25] Upon simultaneous application of VEGF and fumagillin (0.15 nM) we found a nearly complete inhibition of the biosynthesis of both proteins (Figure 2). As expected, fumagillin also inhibits in vivo angiogenesis in the CAM assay (Figure 3).

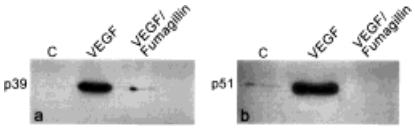


Figure 2. Inhibition by fumagillin (**3**) of VEGF-induced Ets-1 expression in cultured human umbilical vein endothelial cells (HUVEC). The HUVE cells were cultured in Medium 200 (TEBU) with low serum growth supplement (LSGS, TEBU). After confluence, cells were maintained in LSGS-free medium for four hours and then stimulated for four hours with VEGF (150 ng) with or without fumagillin (0.15 nM). Proteins were extracted with Trizol,[34] separated electrophoretically by means of SDS-PAGE, and blotted onto a nitrocellulose membrane. Ets-1 proteins were demonstrated by ELISA. For the detection of Ets-1 proteins a polyclonal rabbit antibody (directed against amino acids 422–441, Santa Cruz, CA) and a monoclonal mouse antibody (directed against amino acids 122–288, Transduction Laboratories) were used. Bands were visualized with the BM Chemoluminescence Western Blotting Kit (Boehringer Mannheim, Germany). VEGF strongly induced both p39 (a, polyclonal rabbit antibody) and p51 (b, monoclonal mouse antibody) Ets-1 proteins (lane 2 in (a) and (b)). This induction was nearly completely inhibited by fumagillin (lane 3).

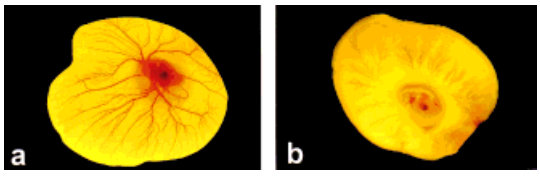
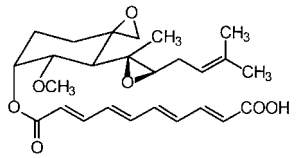


Figure 3. Inhibition of blood vessel development on the chick chorioallantois membrane (CAM) by fumagillin (**3**). A methylcellulose plate[19] (diameter about 2 mm) containing 10 μg of fumagillin was applied on the outer one-third of the CAM on day 5 of development. The results were evaluated after 48 h. Fumagillin caused a significant and generalized inhibition of angiogenesis (b) compared to the negative control (a).

The bisepoxide **3** is one of the most potent angiostatic agents. Its synthetic analogue TNP-470 **4** was the first angiostatic drug in clinical trials.[26]



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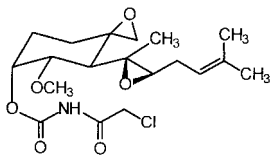

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It has already been shown that fumagillin is a covalent inhibitor of the enzyme methionine aminopeptidase type 2 (MetAP-2).[27] The crystal structure of the fumagillin–MetAP-2 complex has recently been published.[28] However, the link between MetAP-2 inhibition and the angiostatic effect is not yet clear. The cobalt-dependent enzyme MetAP-2 is responsible for the removal of N-terminal methionine residues from specific proteins,[29] which are essential for cell-cycle progression of endothelial cells. It has been supposed that these proteins participate in the signal transduction cascade triggered by VEGF.[30] Our results suggest that inhibition of Ets-1 expression by fumagillin is an important aspect of the angiostatic effect of this natural compound. The way in which this inhibition is achieved is not yet clear. Both a fumagillin-mediated decrease of Ets-1 biosynthesis or a stimulation of Ets-1 degradation are conceivable. In addition, MetAP-2 might partcipate in the regulation of Ets-1 transcription factor activity.[31] In this context it is noteworthy that Ets-1 contains a N-terminal methionine group[32] and is therefore a potential MetAP-2 substrate.

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A Robust, Environmentally Benign Catalyst for Highly Selective Hydroformylation**

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The hydroformylation of olefins to aldehydes is an important example of an efficient and clean process, because of its potentially 100 % atom economy.[1] In the industrial production of C_4 and C_5 aldehydes, where regioselectivity towards the more valuable linear aldehyde product is critical, rhodium – triphenylphosphane complexes are used as catalysts. The products are separated from the catalyst by distillation, which results in catalyst decomposition as an undesirable side reaction. Furthermore, distillation techniques are not suitable for the production of heavier products or fine chemicals because of the high boiling points.

The use of an aqueous biphasic system, in which the water phase contains the dissolved catalyst, affords a straightforward separation of the organic products. To this end, a process using the water-soluble catalyst $[HRhCO(TPPTS)_3]$ (TPPTS = triphenylphosphanyl trisulfonate) has been developed by Ruhrchemie/-Rhône-Poulenc for the hydroformylation of propene.[2] This process meets all the requirements for an environmentally benign process. The applicability of the aqueous biphasic system is, however, strictly limited to substrates that are slightly soluble in water, such as propene and but-1-ene.

A widely investigated approach to facilitate catalyst – product separation is the attachment of the catalyst to a polymeric resin.[3] To date, immobilized catalysts of industrial importance are still unknown: Metal leaching[4] and low catalyst selectivity are the insurmountable problems.

The hydroformylation catalyst that we present here is covalently anchored to a silicate matrix by the sol – gel technique.[5] This material can be prepared by a simultaneous cocondensation of tetraalkoxysilanes and functionalized trialkoxysilanes [Eq. (1)].[6] The sol – gel technique is an ideal method to immobilize catalysts because of its diversity[7] and

$$n\,Si(OR)_4 + 4n\,H_2O \rightarrow n\,Si(OH)_4 + 4n\,ROH \rightarrow \text{polysilicate} \qquad (1)$$

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