The Transcription Factor ETS-1: Its Role in Tumour Development and Strategies for its Inhibition

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Abstract: Transcription factors are an important group of proteins. Changes in expression or activity of transcription factors result in diverse and manifold effects on the whole transcriptome of the cell. Therefore transcription factors are of special interest in physiological as well as pathological processes particularly tumour development and progression. In this review we focus on Ets-1, the prototype of the ETS family of transcription factors. ETS family members play important roles in development, differentiation and proliferation of cells in general and they are involved in apoptosis and tissue remodelling as well. Most of them are downstream nuclear targets of Ras-MAP kinase signalling and the deregulation of *ets* genes results in malignant transformation of different cells. Several *ets* genes are rearranged in human leukaemia, Ewing tumours and prostate cancer to produce chimeric oncoproteins. Furthermore, an aberrant expression of several *ets* genes is often observed in various types of human malignant tumours. With regard to the involvement of some ETS transcription factors, especially Ets-1, in malignant transformation and tumour progression (including invasion, metastasis and neoangiogenesis) through transactivation of cancer related genes, they are potential molecular targets for selective cancer therapy. In this review we focus on the roles of Ets-1 for tumour development and progression with special emphasis on tumour vascularization and invasion. We then discuss specific strategies for Ets-1 inhibition as a potential tool for cancer treatment.

Key Words: Transcription factor, Ets-1, angiogenesis, antiangiogenesis, tumour progression, soybean, tempeh.

ETS-1 AND THE ETS TRANSCRIPTION FACTOR FAMILY

The *ets-1* gene has first been cloned and characterized as the cellular proto-oncogene of the retroviral *v-ets* oncogene of the avian leukaemia retrovirus E26 which induces together with *v-myb* a mixed erythroleucemia in chicken [1, 2]. In further studies Ets-1 turned out the prototype of a novel transcription factor family, the ETS family, which currently includes about 30 members [3, 4]. All of them share a specific DNA-binding domain, called ETS domain, which consists of approximately 80 amino acids with four tryptophane repeats [5] and which binds to double-stranded DNA containing a GGAA/T core motif and different flanking sequences [3, 6-8]. Transactivation as well as transrepression of many target genes can be mediated through ETS transcription factors [9-16]. DNA-binding specificity is determined by the sequences flanking the GGAA/T core and by binding of further transcriptional partners [5, 17]. Transactivation or transrepression can be regulated by interaction with other proteins, by phosphorylation of internal regulatory domains of specific Ets factors *via* protein kinases or Ca^{2+} dependent signalling and finally by acetylation or sumoylation [18-21]. Proteins interacting with ETS factors include other key transcription factors such as AP-1, SP-1, NFκB, CREBP-binding protein/p300, Pax or a combination of different ETS family members [3, 19, 22, 23]. In regulatory regions of genes responsive to Ras signalling an ETS binding site is often found adjacent to an AP-1 binding site [24]. Many different functions have been attributed to ETS family members in different cell types and organisms at molecular and cellular levels, including proliferation, apoptosis or the regulation of matrix degradation [6, 8, 25-45]. Furthermore several *ets* genes are involved in chromosomal translocations leading to the production of chimeric fusion proteins that are associated with various leukemias and soft-tissue cancers [46-54].

EXPRESSION AND ROLES OF THE ETS-1 TRANSCRIPTION FACTOR

Among ETS family members Ets-1 has been most extensively studied and found to be expressed in a broad variety of different cells types where it plays a number of roles for both physiological and pathological processes (Table **1**). Some major findings will be presented in the following sections.

ETS-1 EXPRESSION DURING NORMAL EMBRYOLOGICAL DEVELOPMENT

Since *v-ets* and *v-myb* oncogenes of the oncogenic E26 retrovirus induce a mixed erythroleucemia in chicken, Ets-1

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Table 1. Ets-1 Expression in Physiological and Pathophysiological Processes

Physiological or Pathological Process	Reference
Embryonic development	[34, 55-57]
Vascular development/ Angiogenesis	[40, 58-63]
Hematopoietic differentiation	[34, 55, 64]
Lymphoid differentiation	[5, 65-69]
Neuronal differentiation	[34, 70]
Osteogenic differentiation	[71, 72]
Transcription factor regulation	[73-77]
Pituitary gland/hormone production	[78-80]
Cytokine and growth factor expression	[81-85]
Influence pro-/anti-apoptosis	[42, 86-89]
Viral transformation and viral transcription	[5, 84, 90-92]
Atherosclerosis	[93]
Rheumatoid arthritis	[94, 95]
Breast cancer	[28, 96-99]
Ovarian cancer	[27, 100-102]
Thymoma	[103, 104]
Gastric carcinoma	[105, 106]
Colorectal carcinoma	[48, 107, 108]
Angiosarcoma	[109]
Astrocytic tumours	[70, 110-112]
Thyroid tumours	[104, 113, 114]
Pancreatic carcinoma	[115]
Pulmonary carcinoma	[116, 117]
Chondrosarcoma	[118]
Meningioma	[111]
Endometrial carcinoma	[101, 119]
Esophageal cancer	[106, 120, 121]
Hepatocellular carcinoma	[122, 123]
Oral carcinoma	[124-126]
Bile duct carcinoma	[123, 127]
Prostate cancer	[128-131]
Melanoma	[38, 132, 133]

expression has first been investigated during normal chicken embryogenesis [134, 135]. Expression has been found to be restricted to specific developmental stages and processes [134]. Earliest expression has been observed in hematopoetic islands of the yolk sac. During further development transcripts were demonstrated in endothelial cells during development of blood vessels by both angiogenesis and vasculogenesis [136]. Physiological invasive processes are accompanied by Ets-1 expression as well as the dispersion of somites into the mesenchymal sclerotome, migration of neural crest cells, the branching of epithelial tubules during breast development or the outgrowth of renal tubules from the Wolffian ducts [137-139]. In the latter case Ets-1 is expressed in mesenchymal cells around outgrowing renal tubules. During breast development Ets-1 transcripts are found in the epithelial cells of mammary buds when these first appear [34, 140]. During postnatal mammary gland development Ets-1 expression is highest at the onset of puberty and during early pregnancy, times of extensive epithelial outgrowth and branching. Ets-1 is then expressed in both epithelial cap cells of terminal end buds and in differentiated myoepithelial cells of ducts and alveoli [140]. Functional data concerning Ets-1 expression in normal epithelial cells have shown that Ets-1 is a target of the Scatter factor/hepatocyte growth factor (SF/HGF) signalling through the met receptor tyrosine kinase [137, 141-143]. Normal epithelial MDCK cells disperse and are subject to over-expression of Ets-1 together with several proteases after stimulation by SF/HGF. Other studies have shown that Ets-1 expression was correlated to morphological changes in MDCK cells induced by the SF/HGF activation of a RAS-RAF-MEK-ERK pathway which activates Ets-1 [137, 142-144].

During *in-vivo* embryogenesis the *ets-1* gene is likewise transcribed in migrating cells or along their migration pathways [145]. In the neural crest Ets-1 was found to be upregulated approximately 4-6 hours prior to commencement of neural crest cell migration [146]. It is suggested that Ets-1 orchestrates modifications of cellular adhesions of migrating cells *via* expression of cadherins and integrins as well as the degradation of the extracellular matrix (ECM) through regulation of expression of different proteases [145].

IMPLICATIONS OF ETS-1 IN NEW BLOOD VESSEL FORMATION

Angiogenesis is a conserved process in vertebrates, which is essential for many physiological and pathological processes [147]. Under physiological conditions it occurs during embryological development and in postnatal life during wound healing, tissue regeneration and the menstruation cycle in the female reproductive system [148]. Under normal conditions new blood vessel formation is limited in time due to a finely regulated balance between activator and inhibitor systems. Vascular endothelial growth factor (VEGF) is considered the key angiogenic factor inducing the mitogen-activated protein kinase signalling pathway through receptor tyrosine kinases [149-156]. Under pathological circumstances, e.g. in inflammatory diseases and in particular during tumour development, angiogenesis is unlimited in time and characterized by a proliferative activity of endothelial cells [148]. It is an important prerequisite for tumour development and progression [149, 157-162].

The regulation of angiogenesis under both physiological and pathological conditions has been linked to the Ets-1 transcription factor [25, 134, 136, 163-166]. Descriptive findings

of Ets-1 expression during active new blood vessel formation have subsequently induced functional studies. It has been found that Ets-1 is induced in endothelial cells by direct and indirect angiogenic factors such as VEGF, basic fibroblast growth factor (bFGF) and tumour necrosis factor α (TNF-α) (Fig. (**1**)) [167, 168]. Temporally induced Ets-1 expression is followed by the expression of genes involved in the degradation of the ECM, such as those encoding urokinase-type plasminogen activator (uPA) and different matrix degrading metalloproteinases (MMPs), necessary for endothelial cell invasion during early steps of angiogenesis [166]. Genes involved in cell attachment and cell migration such as integrins, cadherins and ICAMs are controlled by Ets-1 likewise [169-171]. Other factors of the large ETS family are suggested to activate integrin genes in endothelial cells as well [71, 172]. In accordance with these *in-vitro* results we found that application of an Ets-1 antisense oligonucleotide against the AUG start codon and a short downstream sequence is able to effectively inhibit *in-vivo* angiogenesis in the chicken chorioallantoic membrane (CAM) assay [173]. *In-vitro* treatment of human endothelial cells (and vascular smooth muscle cells) with antisense oligodesoxynucleotides against Ets-1 decrease expression of VEGF, HGF and c-met [174-176].

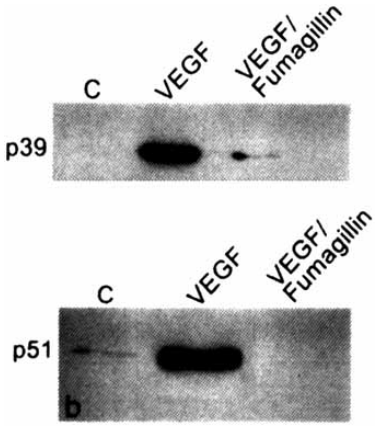

Fig. (1). Inhibition by fumagillin of VEGF-induced Ets-1 expression in cultured human umbilical vein endothelial cells (HUVEC).

HUVEC cells were stimulated for four hours with VEGF (150 ng) with or without fumagillin (0.15 nM). Proteins were extracted, separated by SDS-PAGE electrophoresis and transferred onto nitrocellulose. For the detection of Ets-1 proteins a polyclonal rabbit antibody (directed against amino acid 422-441; Santa Cruz) and a monoclonal mouse antibody (directed against amino acid 122-288; Transduction Laboratories) were used. Bands were visualized with the BM Chemoluminescence Western Blotting Kit (Boehringer Mannheim). 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 in (a) and (b)). This picture was previously published in "Angewandte Chemie International Edition English" [173] and is reprinted by permission of Wiley-VCH Verlag GmbH & CoKG ©1999.

Inhibition of signal transduction upstream of Ets-1 by small molecules or natural compounds is another potential way to restrain angiogenesis. Though this inhibition is not direct (and probably not 100% Ets-1 specific) it can be achieved without transforming cells and also be applied *in-vivo*. We found that the well known antiangiogenic compound fumagillin [177-179] strongly decreases VEGF-induced Ets-1 expression in cultured endothelial cells (Fig. (**1**)) suggesting that an Ets-1 blockade might participate to the antiangiogenic properties of fumagillin [173]. Another angiogenesis inhibitor is genistein which is a well known tyrosine kinase inhibitor [180-183]. Genistein is an isoflavone (5,7,4'-trihydroxyisoflavone), that can be isolated from tempeh, a popular food in south east Asian countries derived from soybeans. We have shown that tempeh contains other isoflavones than genistein such as daidzein (7,4'-dihydroxyisoflavone) and factor2 (6,7,4'-trihydroxyisoflavone) [184] and two novel isoflavones (7,8,4'-trihydroxyisoflavone or 7,8,4'-TriOH and 5,7,3',4'-tetrahydroxyisoflavone or orobol) have been isolated in our laboratories (Fig. (**2**)). The latter are biotransformation products of genistein and daidzein and produced during fermentation of soybeans by microorganisms [185]. We found that all these isoflavones inhibit (like fumagillin) VEGF-induced Ets-1 expression and also proliferation of endothelial cells *in-vitro* in a dose dependent manner [168]. Moreover, all compounds were able to block angiogenesis *in-vivo* in the CAM assay (Fig. (**3**)) [168]. Thus, among molecular mechanisms underlying anti-angiogenic effects of isoflavones could be an inhibition of Ets-1 [168].

Isoflavones have already been proposed to be beneficial for human health due to their hypolipidemic, antiproliferative and antioxidative activities [186-189]. Interestingly, reactive oxygen species, which induce migration and proliferation of cultured endothelial cells, also induce Ets-1 [175]. Inhibition of both Ets-1 expression and endothelial cell proliferation during angiogenesis by isoflavones could therefore be due to their tyrosine kinase inhibitory as well as to their antioxidative effects. Structural modifications might further improve antiangiogenic effects of isoflavones, which have the potential to become useful tools for the treatment of angiogenesis-related diseases. Evidence from both *in-vitro* and *in-vivo* studies is growing that soy isoflavones, in particular genistein, are promising agents for growth inhibition of different human cancers [190]. Genistein has already been reported to be involved in prevention of hormone-related cancers, such as those of the breast and prostate [191-195].

EXPRESSION AND ROLES OF ETS-1 IN TUMOURS

In tumours Ets-1 can be expressed by three cell types: by neoplastic cells, by endothelial cells and stromal fibroblasts of the tumour stroma which represents the tumour microenvironement and which exerts multiple roles during tumour development and progression [43]. In the stroma we found Ets-1 expression within endothelial cells during tumour angiogenesis and within stromal fibroblasts known to contribute to tumour invasion through the secretion of different proteases involved in matrix degradation [28, 30, 39, 136, 166].

ETS-1 EXPRESSION DURING TUMOUR ANGIOGENESIS

Tumour angiogenesis already starts at early stages of tumour development when the tumour microenvironment is exposed to an increased ratio of pro- versus anti-angiogenic

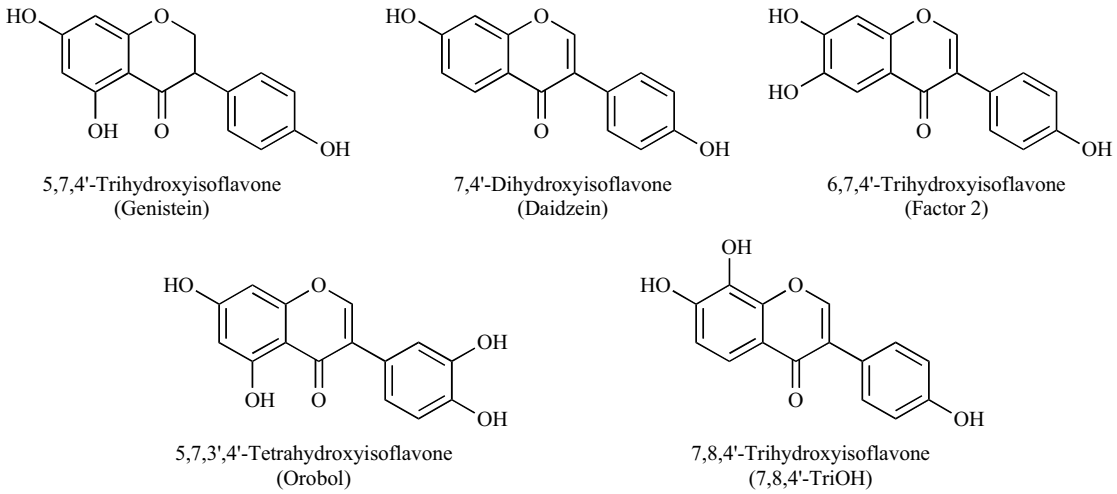

Fig. (2). Chemical structures of tempeh isoflavones.

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factors inducing the so-called angiogenic switch [196]. Pro-angiogenic proteins include VEGF, placenta-like growth factor (PLGF), platelet-derived endothelial growth factor (PDGF), bFGF and angiopoietin-2 (Ang-2). These factors are not only secreted by tumour cells but can also be produced by stromal cells such as PDGF stimulated pericytes [197]. We found that Ets-1 expression within endothelial cells is not restricted to embryogenesis and inflammatory conditions such as rheumatoid arthritis [95] but also regularly seen during vascularization of both benign and malignant tumours [26-28, 43, 136, 166]. During tumour development Ets-1 transcription is already up-regulated in capillaries around pre-invasive stages [28].

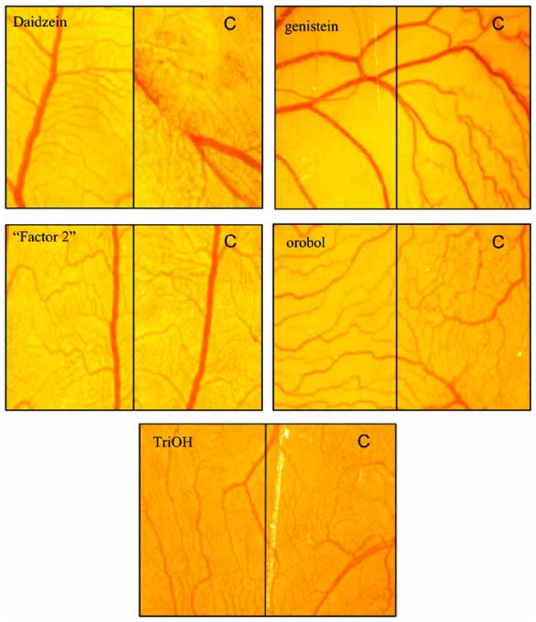

Fig. (3). Examples of inhibitory effects of different isoflavones on *in-vivo* angiogenesis in the chicken chorioallantoic membrane (CAM) assay.

7 to 10 eggs/isoflavone (one platelet/egg) were used to determine antiangiogenic effects. 6 to 10 randomly-selected regions were photographed (using a Wild Makroskop M420 - magnification 100-fold) to show blood vessels beneath the platelets and in a neighbouring control area without platelets. Platelets without isoflavones served as a negative control. Chorioallantoic blood vessels are visible beneath the transparent platelets. Note in Control (C) the presence of an increased number of blood vessels. On the contrary in the region treated with the effective isoflavones genistein and orobol, only very few new blood vessels can be seen. This picture was previously published in "British Journal of Nutrition" [168] and is reprinted by permission of Cabi Publishing ©2005.

Anitangiogenesis is currently emerging as a novel tool for cancer treatment. Strategies include interference with angiogenic ligands, their receptors and downstream signalling, up-regulation of endogenous inhibitors, application of integrin antagonists or inhibitors of MMPs necessary for endothelial cell migration and invasion [198-201]. A dominant negative approach against Ets-1 using retroviral expression of the Ets-1-DNA-binding domain lacking the transactivation domain (Ets-DB) has been successfully applied to inhibit neo-angiogenesis *in-vivo* [165]. Ets-DB is a competitive inhibitor of wild type Ets-1 at promoters of Ets-1 target genes. Intravenous injection of a retroviral expression vector for Ets-DB not only inhibited local bFGF-induced new blood vessel formation in mouse ears but also tumour-induced neo-angiogenesis in mice [165]. Since no severe side effects were observed in other organs (including quiescent blood vessels) a similar approach might prove useful in human cancer treatment.

EXPRESSION AND ROLES OF ETS-1 IN THE FIBROBLASTIC TUMOUR STROMA

Ets-1 expression is not only seen in mesenchymal cells around physiologically invading epithelial structures in the embryo [135] but analogously also in stromal fibroblasts which surround invasive tumour formations (Fig. (**4**)) [166]. Using a two-chambered cell culture system we found that this expression is induced by factors which are secreted by tumour cells towards a stroma-orientated direction [202]. We

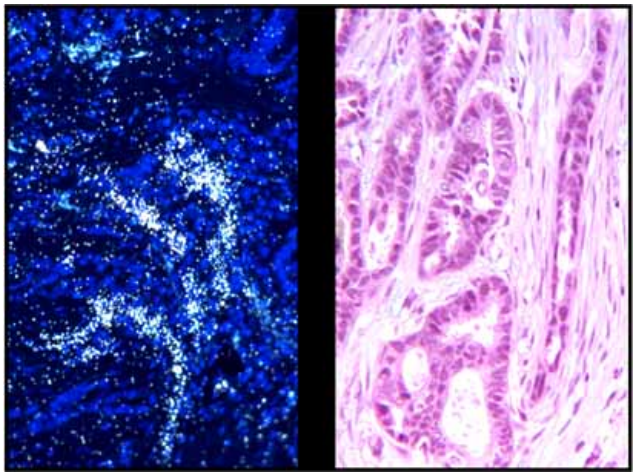

Fig. (4). Expression of Ets-1 in stroma fibroblasts of a human invasive ductal breast cancer.

(A) Ets-1 expression is demonstrated by *in-situ* hybridization using a radiolabelled (^{35}S) probe (autoradiography and darkfield illumination after fluorescent counterstain of the nuclei with Hoechst 33258). (B) Conventional staining (hematoxylin & eosin, H&E) of an adjacent section. Invasive glandular carcinoma formations and spindle stromal fibroblasts between them are discernable.

also identified several factors and cytokines (such as bFGF and PDGF) inducing Ets-1 in cultured fibrobasts [166, 203].

According to co-transfection assays and promoter deletion studies genes encoding several MMPs, such as collagenase IV (MMP 2 and 9), stromelysin 1 (MMP 3) and uPA (which initiates the cascade of proteolytic activation of these enzymes), are among Ets-1 target genes [35, 204-206]. We have studied this regulation in cultured fibroblasts. We found that the same factors which induce Ets-1 in cultured human foreskin fibroblasts (HFF) also induce several of the target genes [166]. We further compared expression of MMPs and their negative regulators (TIMPs, tissue inhibitors of metalloproteinases) between Ets-1 -/- embryonal fibroblasts (isolated from an Ets-1 knock-out mouse) and Ets-1 +/+ wild-type fibroblasts [55, 207]. After stimulation of cells with physiological doses of bFGF (known to induce different proteases and to be expressed by tumour cells) we found that basal Ets-1 levels are not only necessary for a fast induction of MMP 2, 3, 9 and 13 but also for a maintenance of bFGF-induced expression of TIMP 1, 2 and 3 which not only can inhibit MMPs but also participate to an activation of certain pro-MMPs. bFGF induced rapid decrease of TIMP expression in Ets-1 -/- cells could be mediated through a transrepressor antagonized by basal Ets-1 activity (Fig. (**5**)) [207]. *In-vivo* we could finally demonstrate a topographical co-localization of Ets-1 and its proteases encoding target genes in the fibroblastic stroma of different invasive human tumours fitting in well with a regulatory role of Ets-1 for proteases expression in stromal fibroblasts during tumour invasion [26-28, 46, 166].

We finally used substractive suppression hybridization (SSH) between bFGF stimulated wild-type HFF and HFF cells with a reduced Ets-1 expression (HFF Ets-1 invers) in both directions in order to identify novel Ets-1 target genes. We found a number of novel genes that are either activated or repressed by Ets-1 (Hahne *et al*., manuscript in preparation). Most of them belong to genes encoding components of the ECM. Therefore Ets-1 seems not only to be involved in the degradation but also in the molecular composition of the ECM (Hahne *et al*., manuscript in preparation).

EXPRESSION OF ETS-1 IN TUMOUR CELLS

Among physiologically migrating and invading Ets-1 expressing cells in embryos are branching epithelial structures during breast development and also neural crest cells. This prompted us to study Ets-1 expression in corresponding tumours.

Melanocytes derive from the neural crest and we found a strong up-regulation of Ets-1 transcripts during development of malignant melanomas [38]. While normal human melanocytes and benign melanocytic lesions (such as melanocytic naevi) are Ets-1 negative, an increasing up-regulation of Ets-1 expression is seen from *in-situ* melanomas towards invasive tumours and melanoma metastases (Fig. (**6**)) [38]. The functional roles of Ets-1 in melanoma cells was addressed by using RNA intereference (RNAi) to reduce gene expression [208-211]. Decreased Ets-1 levels significantly reduced proliferation, anchorage independent growth and invasion of melanoma cells, all of which are typical features of the neoplastic phenotype [30, 38]. Tumour cell invasion requires both matrix degradation and cell migration through the ECM mediated by interactions of tumour cell integrins with the ECM. In accordance down-regulation of Ets-1 resulted in melanoma cells in diminished expression of Ets-1 target genes encoding MMP 1, 3, uPA and integrin β3 [30, 38]. This gene belongs to a group of integrin genes (integrin-αIIb, -αV, -β2, -β3 and -β4) which contain ETS-binding sites in their promoters [172, 212-215].

Glia cells are neural tube-derived cells as well and gliomas are the most frequent malignant human brain tumours. Rat C6 glioma cells are a current model to study gliomas and we found that these cells likewise express Ets-1. We used a so-called decoy strategy to inactivate Ets-1 in C6 glioma cells. Decoy oligodesoxynucleotides (ODNs) are short 20-24mer double stranded DNA sequences which contain binding sites of transcription factors resulting in an attenuation of *cis-trans* interactions with subsequent modulation of gene expression. Transfection of C6 glioma cells with an ODN containing the consensus DNA-binding sequence of Ets-1 resulted in a decrease of cell proliferation and in reduced expression of the Ets-1 target gene *mmp-9* [39, 216]. Thus decoy ODNs likewise might be useful experimental tools for glioma therapy. Problems are their cell type unspecific action and their intracellular degradation which started 24 h after transfection in our experiments [39]. Our results are comparable to findings obtained with a CRE decoy ODN in MCF-7 cells [217].

In further experiments a transdominant negative approach was applied to block Ets-1 in rat C6 glioma cells using retroviral expression of the Ets-DB as described above for endothelial cells [139, 165, 218]. C6 glioma cells stably expressing Ets-DB show a reduced proliferation and anchorage independent growth in soft-agar as well as a diminished migra-

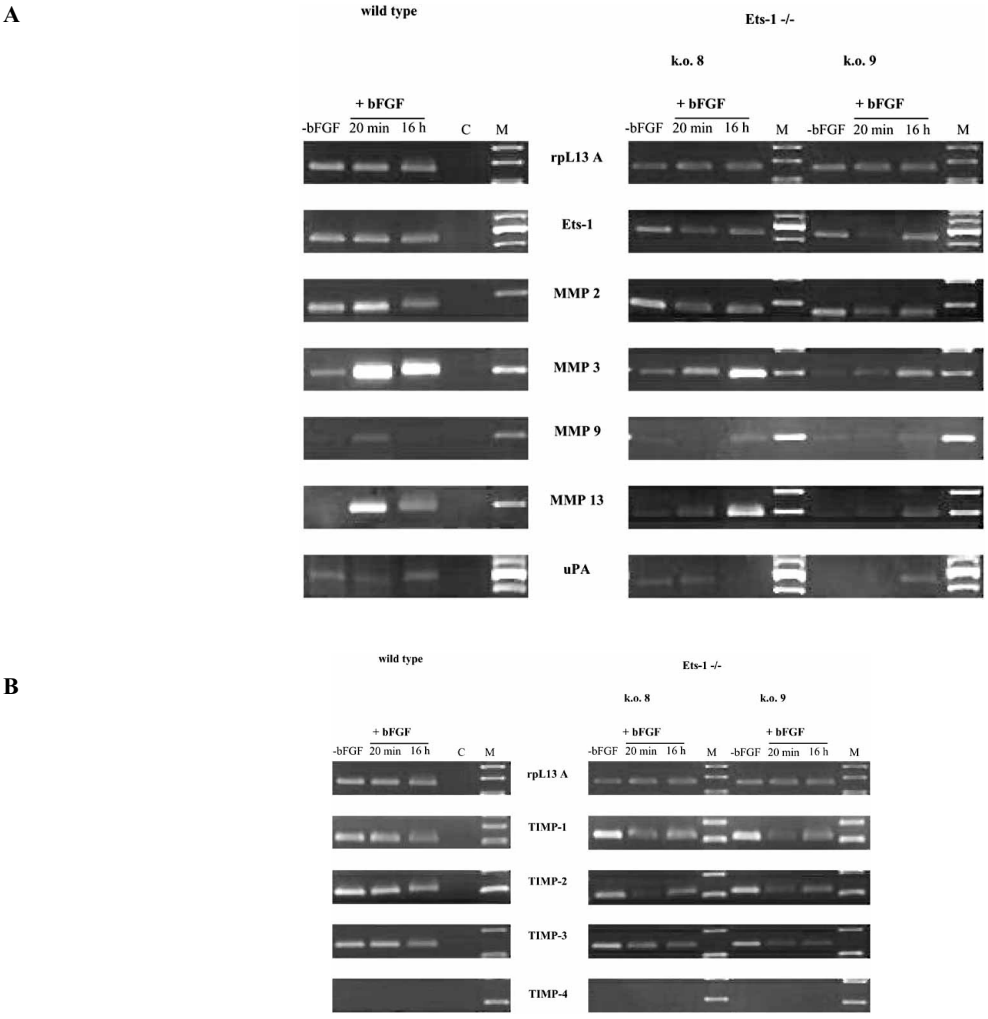

Fig. (5). RT-PCR analysis of Ets-1 and target genes in wild type fibroblasts compared to Ets-1 -/- fibroblasts.

Expression of transcripts was analysed in 2 % agarose gels in wild type and Ets-1 -/- fibroblasts derived from two different animals (k.o. 8 and k.o. 9) without and after induction with bFGF (10 ng/ml) for 20 min and 16 hours. The negative control (C) for any PCR is shown at the end of every line. **A**) RT-PCR analysis of metallomatrix proteinases (MMPs). It is evident that *ets-1* transcripts are not induced by bFGF and that basal *ets-1* levels are necessary for early induction of MMP 2, 3 and 13. Fragment sizes were evaluated with a PCR-marker (M); gene specific products are 251-bp for rpL13A, 536-bp for Ets-1, 166-bp for MMP 2, 280-bp for MMP 3, 188-bp for MMP 9, 295-bp for MMP 13 and 525-bp for uPA. **B**) RT-PCR analysis of tissue inhibitors of metallomatrix proteinases (TIMP1-4). Basal *ets-1* levels are required for a maintainance of TIMP-1 to -3 expression. Gene specific products are 251-bp for rpL13A, 108-bp for TIMP-1, 122-bp for TIMP-2, 135-bp for TIMP-3 and 130-bp for TIMP-4, evaluated by PCR-marker (M). This picture was previously published in "International Journal of Molecular Medicine" [207] and is reprinted by permission of D.A. Spandidos©2006.

tion and again a decreased expression of the Ets-1 target gene *mmp-9*. In accordance with these *in-vitro* findings, Ets-DB expressing C6 glioma cells exhibited a reduced tendency to develop tumours in the CAM assay *in-vivo* compared to wild-type C6 cells (Sahin *et al*., manuscript in preparation).

In epithelial carcinoma cells *ets-1* expression is less frequently found than in other tumour cells or in stromal cells [27, 28, 136, 166]. Since little is known about the biological role of Ets-1 in epithelial tumour cells we addressed this issue using the neoplastic epithelial HeLa cell line likewise expressing Ets-1 [30, 219]. We found that Ets-1 enhances in HeLa cells anchorage-independent growth, a hallmark of transformation (Fig. (**7**)) [30]. Moreover Ets-1 over-xpression activates the *mmp 1* promoter and increases integrin-β2 expression [30]. In contrast, down-regulation of Ets-1 by an antisense strategy resulted in diminished expression of genes encoding MMP 1 and 3, uPA as well as integrin-β2 [30, 38]. All these genes are involved in the invasive phenotype of Hela cells.

Using *in-situ* hybridization we finally found a focal expression of Ets-1 *in-vivo* in the neoplastic cells of about 10% of invasive human breast cancers. In human breast cancer cell lines Ets-1 expression has been suggested to be associated with *in-vitro* invasiveness and epithelial-mesenchymal transition, linked to an expression of vimentin, uPA, MMP 1 and MMP 3 as well as to a loss of E-cadherin [220].

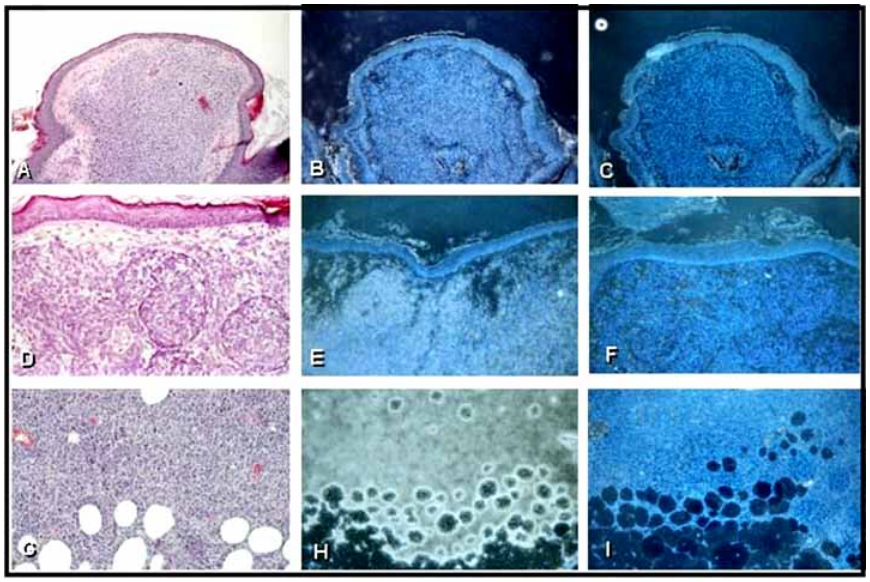

Fig. (6). ***Ets-1 in-situ*** **hybridization of human melanocytic lesions.**

(**A-C**) shows dermal melanocytic nevus, (**D-F**) invasive malignant melanoma of the skin and (**G-I**) metastatic melanoma within peritoneal fat tissue.

Ets-1 transcripts (light grains) are seen in melanocytic nevus (**B**). Expression is strong in invasive malignant melanoma (**E**) and maximal in neoplastic cells of the metastasis (**H**). No positive signals are seen in negative control sections (**C**, **F** and **I**, *ets-1* sense riboprobe). Histomorphology of the lesions is shown in **H&E**-stained slides (**A**, **D** and **G**). Specific hybridization signals and negative controls are demonstrated in darkfield illuminations of the slides after fluorescent counterstaining of the nuclei with Hoechst 33258. Bar = 100µ. This picture was previously published in "Cellular and Molecular Life Science" [38] and is reprinted by permission of Birkhäuser Basel ©2004.

ROLE OF ETS-1 SPLICE VARIANTS

Beside the full length Ets-1 protein several Ets-1 splice variants lacking exon 4, exon 6 (in melanoma cells), exon 7 (in colon and breast cancers) or both exon 4 and 7 (in colon cancers) have been detected [28, 38, 221-224]. The splice variant lacking exon 7 has been shown to rescue Fas-induced apoptosis in colon carcinoma cells [87, 225, 226]. It remains to be investigated whether other splice variants display different functional properties in human tumours.

IN TUMOURS ETS-1 EXERTS SIMILAR ROLES IN THE NEOPLASTIC CELLS AND CELLS OF THE TUMOUR STROMA

The results obtained in different tumours and neoplastic cell lines show that Ets-1 exerts similar roles in different tumour cells as well as in endothelial cells and fibroblasts of the tumour stroma. Ets-1 stimulates cellular proliferation, anchorage-independent growth as well as expression of integrins and proteases involved in cell migration and invasion. All these processes are important for tumour development and progression and for tumour angiogenesis.

MMP production appears to confer to cancer cells a higher metastatic potential [227]. Activated MMP 1 for example plays an important role in invasive properties of a broad variety of cancer cells [26-28, 228] and Ets-1 has been shown to be co-expressed with MMP 1 and uPA in various types of tumours [38, 98, 117]. Breast cancers co-express Ets-1, MMP 1 and MMP 9 [28]. In pancreatic cancer high expression levels of Ets-1, MMP 2 and MMP 9 correlate with poor prognosis [229]. Fitting well with these findings

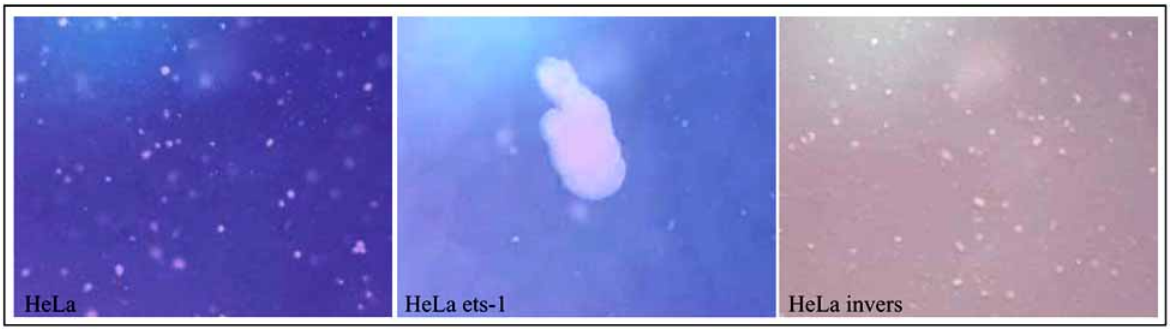

Fig. (7). Ets-1 expression induces anchorage independent growth of HeLa cells.

HeLa, HeLa-Ets-1 and HeLa-invers cells were individualized and cultured in soft-agar. Only the HeLa cells overexpressing Ets-1 formed growing, anchorage-independent colonies. Pictures were taken at day 30 at a 43.75fold magnification. This picture was previously published in "ONCOGENE" [30] and is reprinted by permission of Nature Publishing Group ©2005.

Ets-1 levels have been associated with malignancy grade and prognosis of several tumour types [26, 48, 96, 110-112, 117, 230]. A broad variety of MMPs inhibitiors have already been evaluated in human clinical trials for cancer treatment but results have been unsatisfactory until now [231-234]. Treatment failures are attributed to lack of specificity, toxicity and cancer stages. Moreover, recent studies have demonstrated that some MMPs might also play a protective role during cancer progression. Increased MMP 12 expression by colon carcinoma cells is associated with increased survival [235] and MMP 8 deficient mice display increased skin cancer susceptibility [236]. Thus dual functions of some MMPs during tumour progression can prevent beneficial effects of unselective MMPs inhibitors most of which target all MMPs.

CONCLUSION

In conclusion findings obtained about Ets-1 *in-vitro* and *in-vivo* suggest that this transcription factor exerts manifold effects on tumours by acting both on neoplastic cells and cells of the tumour stroma where Ets-1 participates to the two major roles of stroma, tumour vasczularization and promotion of invasion.

The findings also evoke the possibility that this transcription factor might be a suitable target for tumour treatments directed at both neoplastic and stromal cells.

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