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15-Lipoxygenase-1 Induces Lipid Peroxidation and Apoptosis, and Improves Survival in Rat Malignant Glioma

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Abstract. *Background: Previous studies suggest either an anti- or pro-apoptotic role for 15-lipoxygenase-1 in carcinogenesis. Materials and Methods: We used adenovirus gene transfer of human 15-lipoxygenase-1 to characterize its effects in vitro and in vivo. Results: 15-Lipoxygenase-1 expression in mouse macrophages resulted in a significant, 25-fold, induction in the production of the specific 15-lipoxygenase-1 product 13-hydroxyoctadecadienoic acid. Tail vein gene transfers in mice led to highest expression of the transduced 15-lipoxygenase-1 in liver and spleen. In the liver, 15-lipoxygenase-1 significantly increased lipid peroxidation by 3.5-fold and 2-fold, three and seven days after transduction, respectively. A significant 32-fold induction in caspase-3 activity was detected in 15-lipoxygenase-1 expressing livers seven days after transduction. In a syngeneic rat model of malignant glioma, 15-lipoxygenase-1 extended survival significantly (p=0.001). Conclusion: Our results support the pro-apoptotic role of 15-lipoxygenase-1 and suggest that 15-lipoxygenase-1 could be a potential new target gene for the therapy of malignant glioma.*

Lipoxygenases (LO) are a family of lipid-peroxidizing enzymes catalyzing the introduction of molecular oxygen to polyunsaturated fatty acids. In mammals, they are classified into 5-, 8-, 12- and 15-LOs according to their positional specificity of arachidonic acid oxygenation (1). The primary 15-lipoxygenase-1 (15-LO-1) products from arachidonic acid and linoleic acid are 15(*S*)-hydroperoxyeicosatetraenoic acid and 13(*S*)-hydroperoxyoctadecadienoic acid, respectively (2). These lipid hydroperoxides are readily reduced to their corresponding hydroxy derivatives, namely 15-hydroxyeicosatetraenoic acid and 13-hydroxyoctadecadienoic acid (13-HODE) (1). For 15-LO-1, linoleic acid is the preferred substrate (3), whereas arachidonic acid can also be metabolized by other LOs, cyclo-oxygenases and cytochrome P450 (4). In addition to free fatty acids, 15-LO-1 is also capable of oxidizing more complex substrates, such as biological membranes, phospholipids, cholesterol esters and plasma lipoproteins (1, 2). 15-LO-1 belongs to the class of reticulocyte-type lipoxygenases and is highly expressed in reticulocytes, eosinophils and bronchial epithelial cells (1). In reticulocytes, 15-LO-1 reacts with mitochondrial membrane lipids, initializing the controlled breakdown of mitochondria during the reticulocyte maturation process (5). The physiological role of 15-LO-1 in cells other than reticulocytes remains unknown. 15-LO-1 and its reaction products have been suggested to be involved in many pathological conditions, such as skin and respiratory syndromes in allergic diseases, inflammation, atherogenesis and carcinogenesis (1, 6, 7). Reactive lipid hydroperoxides produced by 15-LO-1 can affect the cellular redox balance and may have various effects on cellular homeostasis and cell signalling.

Extensive evidence from *in vitro* experiments implicate a pro-apoptotic role for 15-LO-1 and its reaction product 13(S)-HODE in colorectal and esophageal cancer cells (8-10). These studies also show that the induction of apoptosis by different kinds of chemopreventive agents, *e.g.* nonsteroidal anti-inflammatory drugs, histone deacetylase inhibitors, and methyltransferase inhibitors, is dependent on the induction of

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15-LO-1 activity and production of 13(S)-HODE. Anti-tumorigenic effects of 15-LO-1 have been shown by mouse models of colorectal carcinoma (11-13), as well as by two different cancer models in transgenic mice overexpressing human 15-LO-1 (14).

To clarify the effects of 15-LO-1 *in vivo*, we first studied the adenovirus mediated gene transfer of human 15-LO-1 in mice. We then tested the effect of 15-LO-1 in a syngeneic rat model of malignant glioma.

Materials and Methods

Production of recombinant adenoviruses. The recombinant Adh15-LO-1 adenovirus and control AdlacZ virus containing nuclear-targeted *Escherichia coli lacZ* gene (15) were produced in 293 Embryonic Kidney cells (CRL-1573, ATCC, Manassas, VA, USA) by homologous recombination as described elsewhere (16).

In vitro transductions, mRNA isolation and Northern hybridization. RAW264.7 macrophages (TIB-71, ATCC, Manassas, VA, USA) were transduced with recombinant Adh15-LO-1 virus and AdlacZ control virus with multiplicity of infection (MOI) of 100. Messenger RNA was isolated 24, 48 and 72 h after transduction and Northern hybridization was performed as described elsewhere (17).

15-LO-1 activity assay. The capacity of the recombinant Adh15-LO-1 virus to produce functionally active 15-LO-1 protein was verified from transduced RAW264.7 mouse macrophages 72 h after transduction. The cells were lysed and incubated with linoleic acid and the formation of hydroxy fatty acids was measured by gas chromatography (17). Specific 15-LO inhibitor PD146176 (18, 19) (a generous gift from Dr. Joseph Cornicelli, Pfizer, Ann Arbor, MI, USA) was tested in some experiments at a concentration of 10 μM. The inhibitor was added 15 min before the linoleic acid incubation.

In vivo gene transfers into mice via tail vein. All animal experiments were approved by the Experimental Animal Committee, University of Eastern Finland. Low density lipoprotein receptor knock-out mice (20) and scavenger receptor knock-out mice (21) (male and female, F8-F10 generations, age 6-12 weeks) were transduced intravenously *via* tail vein injection with 10^{11} virus particles of recombinant Adh15-LO-1 and AdlacZ in a total volume of 200 μl (22). The mice were sacrificed three or seven days after the transduction. Snap-frozen tissue samples from liver, spleen, kidney, lung and aorta were collected for RNA isolation, lipid peroxidation assay and caspase-3 activity analysis, and samples were also processed for immunohistochemistry (22).

Genomic PCR and reverse transcription-PCR. Genomic DNA and total RNA were isolated from snap-frozen tissue samples by TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Distribution of the transferred gene was detected with polymerase chain reaction (PCR) from genomic DNA samples, and mRNA expression of the transferred gene was detected from the total RNA samples with reverse transcription-PCR (RT-PCR) (22). PCR reactions were performed as described (15).

Immunohistochemistry. 15-LO-1 protein expression was detected from paraffin sections with immunohistochemistry using a polyclonal rabbit anti-human 15-LO antibody (23) (1:1000 dilution). Apoptosis was analyzed from paraffin sections using a polyclonal antibody against the active form of caspase-3 (anti-ACTIVE® Caspase-3 pAb; Promega, Madison, WI, USA) (1:250 dilution). Controls, where the primary antibody was omitted, were included in all immunohistochemical stainings (24). Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA, USA) was used in all stainings for visualization (Olympus Optical, Tokyo, Japan).

Lipid peroxidation assay. Lipid peroxidation, an indicator of oxidative stress, was measured from snap-frozen liver samples according to the colorimetric BIOXYTECH® LPO-586™ lipid peroxidation assay (Oxis International, Inc., Portland, OR, USA). The assay is based on the reaction of a chromogenic agent, *N*-methyl-2-phenylindole, with malondialdehyde (MDA) and 4-hydroxyalkenals, which are decomposition products of the unstable lipid peroxides. For the assay, the snap-frozen tissue samples were homogenized in ice-cold 20 mM Tris buffer, pH 7.4, containing 5 mM butylated hydroxytoluene to prevent sample oxidation. The homogenates were centrifuged at 4˚C at 3000 ×g for 10 min to remove large particles. The supernatants were used for the lipid peroxidation assay and for protein determination by Coomassie Plus Protein Assay Reagent Kit (Pierce, Rockford, IL, USA).

Caspase-3 enzyme activity assay. Enzymatic detection of caspase-3 activity in mouse liver samples was performed as described elsewhere (25).

BT4C rat glioma model. BDIX male rats (Charles River Laboratories International, Inc., Wilmington, MA, USA) were injected with 10^4 BT4C cells to a depth of 2.5 mm into the right corpus callosum as previously described (26). The rats were divided into two cohorts: group I, which received Adh15-LO-1 gene therapy (n=12) and group II, which was a control group (n=5) and did not receive any treatment. Tumor growth was verified 14 days after BT4C cell injection by magnetic resonance imaging (MRI). On the following day animals received Adh15-LO-1 (2.7×10^{12} vp/ml) injection to a depth of 1.5, 2.0 and 2.5 mm (2.5 μl per site), as well as two additional injections applied the next day (at 2 mm depth at an angle, anteriorly and posteriorly, made by flexing the C-arm of the microinjection unit). Tumor growth was assessed by MRI again two and four weeks after the treatment. Those animals that did not present tumor on the first MRI, as well as those that had shown extracranial tumor growth, were excluded from the study (n=3). The rest of the animals were used for survival studies. Animals were sacrificed upon human endpoints. Survival was calculated in days from the day of BT4C cell inoculation.

An additional study was performed for histological analysis. Six animals were divided into a group that received Adh15-LO-1 gene therapy (n=3) and a control group (n=3). Gene therapy treatments were performed as described above, with the exception that the tumor growth was verified 21 days after inoculation and then followed by the gene transfer. Blood and serum samples were collected before gene therapy procedure for routine clinical chemistry assays at Kuopio University Hospital. Seven days after the gene transfer, blood and serum samples were withdrawn again and the animals were sacrificed. The brain tissues were fixed overnight in 4% paraformaldehyde solution and paraffin embedded.

Magnetic resonance imaging. MRI was used for tumor visualisation. The rats were anaesthetized with isoflurane (induction 5% and maintenance 1.5% with carrier gas of O_2 30% and N_2O 70%) and

fixed to a stereotactic holder. MRI data were acquired using a horizontal 4.7 T magnet (Magnex Scientific Ltd, Abington, UK) interfaced to a Varian UnityINOVA console (Palo Alto, CA, USA) and using an actively decoupled volume transmission coil and a quadrature surface receiver coil (Rapid Biomedical, Germany). T2-weighted images were measured using a spin-echo sequence (echo time (TE)=40 ms, repetition time (TR)=2 s). A total of 17 slices, each of 1 mm thickness, were imaged. The matrix size was 256X128 zero-filled to 256×256, with field of view (FOV) of 40 mm^2, yielding an in-plane resolution of 156 μm.

Total tumor volume was processed and analysed using Matlab version 7.04 (MathWorks Inc., Natick, MA, USA). Tumor volumes were calculated by delineating the tumor area in all the image slices, using premade macros. To minimize human errors, tumor volumes were measured independently by two individuals (AP and FA) and the mean values of the two measurements are reported. A multiplication factor to convert raw pixel data into tumor volumes (mm^3) was calculated using the following formula: $(FOV\ (mm)/pixel\ size)^2$.

Statistical analysis. All results were analysed in GraphPad Prism Version 4.03 (GraphPad Software Inc., La Jolla, CA, USA). Statistical significance was evaluated using one-way ANOVA followed by Newman-Keuls Multiple Comparison test, or Mann-Whitney test. Survival analysis was performed using Kaplan-Meier log-rank test and tumor volume comparison with unpaired *t*-test. $P<0.05$ was considered statistically significant.

Results

In vitro verification of the functionality of the recombinant Adh15-LO-1 virus. In order to verify that the recombinant Adh15-LO-1 virus produces functional 15-LO-1 protein, *in vitro* transductions of RAW264.7 mouse macrophages were performed. Adh15-LO-1 transduction led to the expression of *15-LO-1* mRNA in RAW264.7 cells 24, 48 and 72 h after transduction, whereas no expression was detected in the control (untransduced) or in the AdlacZ- transduced cells (Figure 1A). Increased 15-LO-1 activity, measured as 13-HODE production after incubation with linoleic acid, was detected from RAW264.7 cells 72 h after transduction (Figure 1B). The production level of 13-HODE was significantly higher (25-fold, $p<0.001$) in the Adh15-LO-1-transduced cells compared with the untransduced or the AdlacZ-transduced cells (Figure 1B). The specific 15-LO inhibitor PD146176 (18) very efficiently prevented 15-LO-1 enzymatic activity (five-fold reduction in Adh15-LO-1-transduced cells *vs*. Adh15-LO-1-transduced cells + PD146176) (Figure 1B). The production levels of the byproducts 2- and 9-HODE did not differ significantly between the groups. The proportion of 13-HODE of all the HODEs detected was 75%.

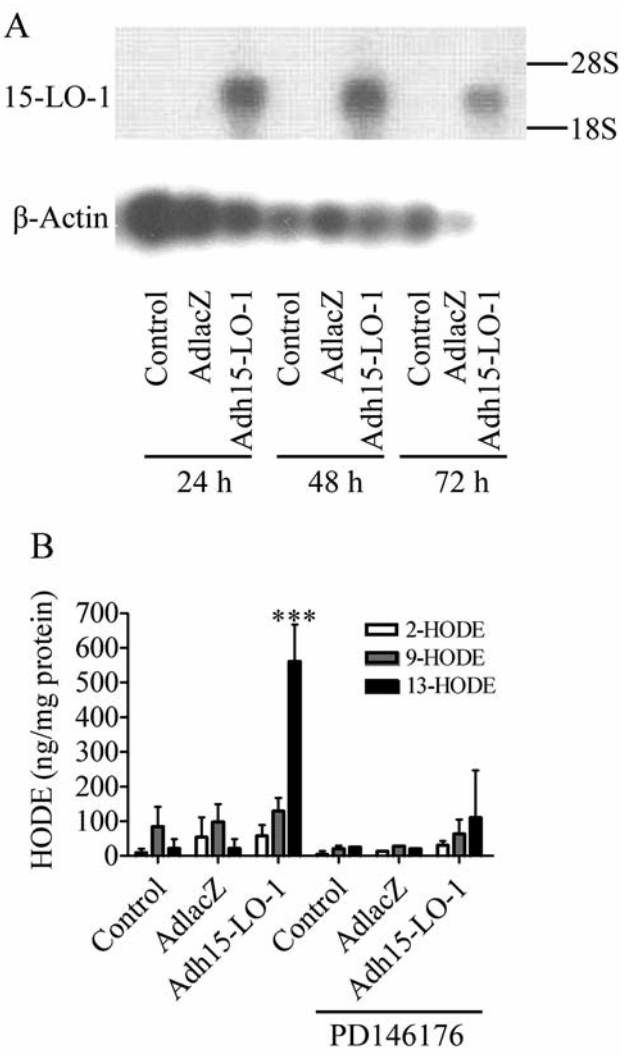

Figure 1. *A: Northern blot analysis of 15-lipoxygenase-1 (15-LO-1) mRNA expression in RAW264.7 cells 24, 48 and 72 h after transduction. The cells were transduced with recombinant Adh15-LO-1 and control AdlacZ adenovirus with multiplicity of infection (MOI) 100. Total RNA was isolated from the transduced and untransduced (control) cells 24, 48 and 72 h after transduction and Northern hybridizations were performed with probes specific for human 15-LO-1 and for rabbit β-actin. B: Gas chromatographic hydroxyoctadecadienoic acid (HODE) analysis from the transduced RAW264.7 cells after incubation with linoleic acid. Untransduced (control), AdlacZ-transduced and Adh15-LO-1-transduced cells were lysed and incubated with 50 μM linoleic acid for 15 min at 37°C. For some experiments, 10 μM 15-LO inhibitor PD146176 was added to the cell lysates 15 min before the linoleic acid incubation. Results are presented as means±SD, n=4/group (for the inhibitor study n=2/group). ***p<0.001 Adh15-LO-1 vs. untransduced and vs. AdlacZ, one-way ANOVA followed by Newman-Keuls multiple comparison test.*

Expression of the transduced 15-LO-1 after tail vein injections in mice. Our previous study with tail vein injections of recombinant adenoviruses into mice showed that the expression of the transduced gene was highest in liver and spleen (22). Therefore, we analyzed the presence of the genomic human *15-LO-1* DNA and the expression of the transduced human *15-LO-1* mRNA by PCR in the liver and spleen after transduction. Genomic human *15-LO-1* DNA (956 bp PCR fragment) was detected three and seven days after Adh15-LO-1 transduction

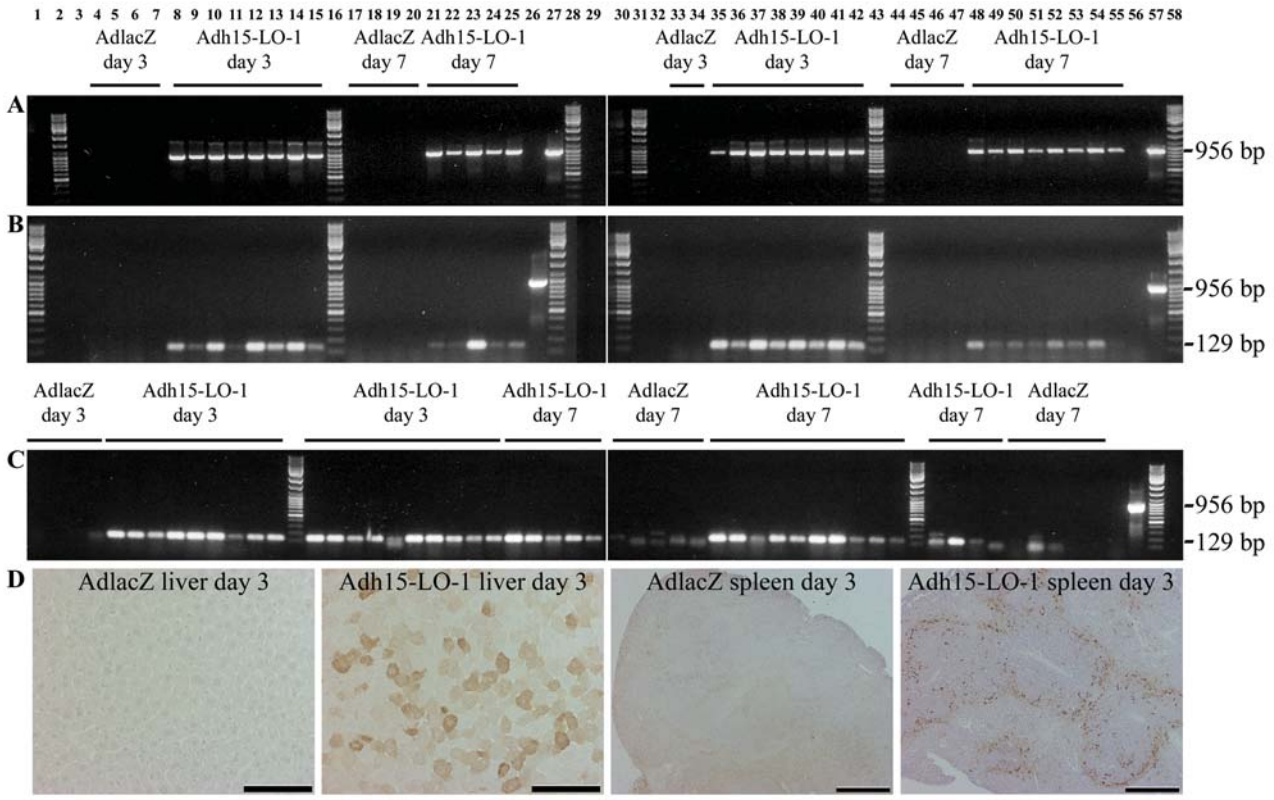

Figure 2. *PCR detection of Adh15-LO-1 genomic DNA (A) and RT-PCR analysis of the mRNA expression of the transduced human 15-LO-1 (B and C) from mice transduced via tail vein with 1×10^{11} virus particles of recombinant Adh15-LO-1 and AdlacZ viruses then sacrificed three or seven days after gene transfer. A: PCR detection of Adh15-LO-1 genomic DNA from mouse liver and spleen. Lane 1, empty; lane 2, molecular weight marker; lane 3, negative control from PCR reaction. AdlacZ day 3: lanes 4 and 6, liver; lanes 5 and 7, spleen. Adh15-LO-1 day 3: lanes 8, 10, 12 and 14, liver; lanes 9, 11, 13 and 15, spleen. Lane 16, molecular weight marker. AdlacZ day 7: lanes 17 and 19, liver; lanes 18 and 20, spleen. Adh15-LO-1 day 7: lanes 21, 23 and 25, liver; lanes 22 and 24, spleen. Lane 26, liver of untransduced mouse; lane 27, positive control; lane 28, molecular weight marker; lanes 29 and 30, empty; lane 31, molecular weight marker; lane 32, negative control from PCR reaction. AdlacZ day 3: lane33, liver; lane 34, spleen. Adh15-LO-1 day 3: lanes 35, 37, 39 and 41, liver; lanes 36, 38, 40 and 42, spleen. Lane 43, molecular weight marker. AdlacZ day 7: lanes 44 and 46, liver; lanes 45 and 47, spleen. Adh15-LO-1 day 7: lanes 48, 50, 52 and 54, liver; lanes 49, 51, 53 and 55, spleen. Lane 56, liver of untransduced mouse; lane 57, positive control; lane 58, molecular weight marker. B: RT-PCR detection of transduced human 15-LO-1 mRNA expression from mouse liver and spleen. Lane 1, molecular weight marker; lane 2, negative control from PCR reaction; lane 3, negative control from cDNA synthesis; lanes 4-25, as in (A) (RNA samples from the same tissues in the same order); lane 26, positive control; lane 27, molecular weight marker, lanes 28 and 29, empty; lane 30, molecular weight marker; lane 31, negative control from PCR reaction; lane 32, negative control from cDNA synthesis; lanes 33-55, as in (A) (RNA samples from the same tissues in the same order); lane 56, liver of untransduced mouse; lane 57, positive control; lane 58, molecular weight marker. C: RT-PCR detection of transduced human 15-LO-1 mRNA expression from mouse liver, spleen, kidney, lung and aorta. AdlacZ day 3: lane 1, liver; lane 2, spleen; lane 3, kidney; lane 4, lung. Adh15-LO-1 day 3: lanes 5, 9, 15 and 20, liver; lanes 6, 10, 16 and 21, spleen; lanes 7, 11, 17 and 22, kidney; lanes 8, 12, 18 and 23, lung; lanes 13, 19 and 24, aorta. Lane 14, molecular weight marker. Adh15-LO-1 day 7: lanes 25, 35, 40 and 46, liver; lanes 26, 36, 41 and 47, spleen; lanes 27, 37, 42 and 48, kidney; lanes 28, 38 and 43, lung; lanes 29, 39, 44 and 49, aorta. AdlacZ day 7: lanes 30 and 50, liver; lanes 31 and 51, spleen; lanes 32 and 52, kidney; lanes 33 and 53, lung; lanes 34 and 54, aorta. Lane 45, molecular weight marker; lane 55; liver of untransduced mouse; lane 56; positive control; lane 57, molecular weight marker. D: Immunohistochemical detection of protein expression of transduced human 15-LO-1 in mouse liver and spleen with a polyclonal rabbit anti-human 15-LO antibody (23) (1:1000 dilution) three days after transduction. Liver: scale bar 50 μm. Spleen: scale bar 200 μm.*

(Figure 2A), whereas no signal was obtained from intact tissues, from untransduced nor from AdlacZ-transduced mice. Expression of the transduced human *15-LO-1* mRNA (129 bp PCR fragment) was most prominent in liver, but also detectable in spleen, both three and seven days after transduction. However, the expression was already diminishing at the later timepoint (Figure 2B). No *15-LO-1* mRNA expression was detected in untransduced nor in the AdlacZ-transduced mice. We also studied the mRNA expression of the human *15-LO-1* in other tissues of transduced mice showing that the expression was highest in liver and spleen, but could also be detected in kidneys, lungs and aorta (Figure 2C).

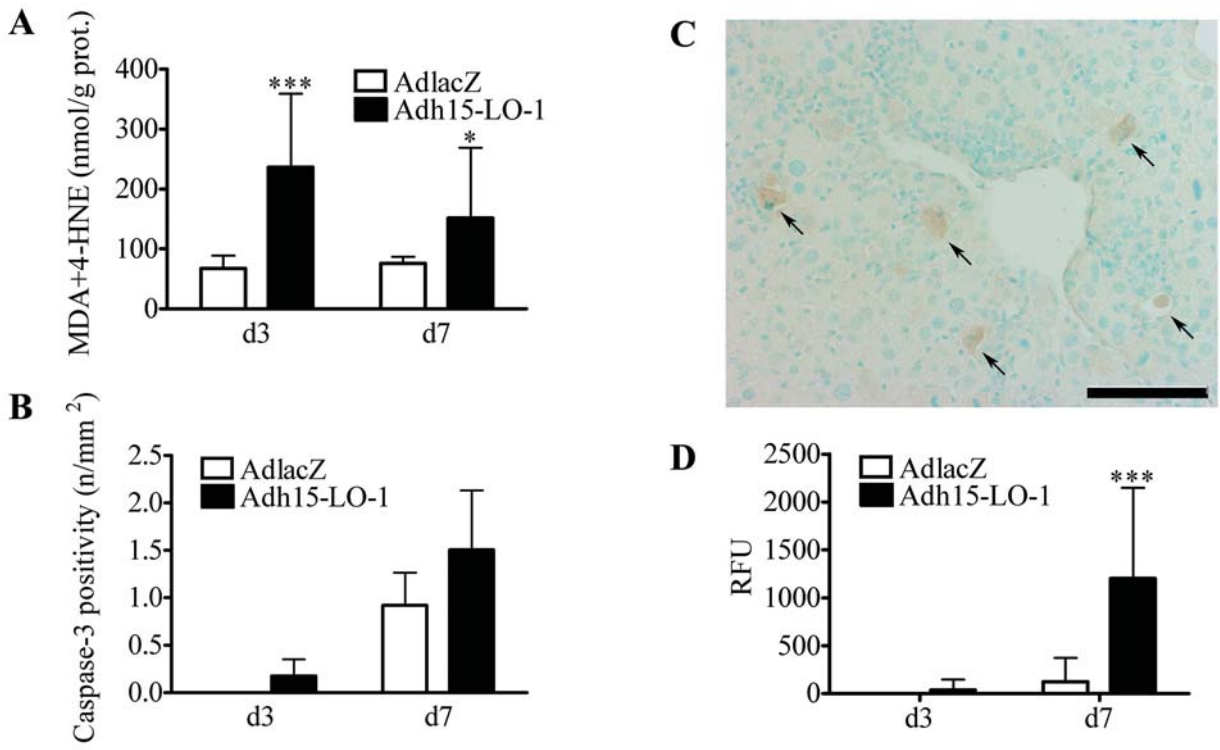

Figure 3. *Analysis of lipid peroxidation and apoptosis from mouse livers three and seven days after gene transfer. The mice were transduced via tail vein with 1×10^{11} virus particles of recombinant AdlacZ or Adh15-LO-1. A: Analysis of lipid peroxidation from the mouse livers by colorimetric lipid peroxidation assay. The results are presented as means±SD, n=8-13/group. ***p=0.003 Adh15-LO-1 vs. AdlacZ; *p=0.0229 Adh15-LO-1 vs. AdlacZ; Mann-Whitney test. Immunohistochemical analysis of the active form of caspase-3 from the mouse livers. Paraffin sections were immunostained with a polyclonal antibody against the active form of caspase-3 using methyl green as the counterstain. B: Quantitative analysis of caspase-3-positive cells (means±SD, n=3-4/group at day 3 and n=7-8/group at day seven). C: Caspase-3-positive hepatocytes (arrows) exhibited typical apoptotic morphology, i.e. rounded shape, condensation of chromatin and cell shrinkage. D: Caspase-3 enzymatic activity was measured from liver homogenates containing 50 μg of protein. The results are presented as mean relative fluorescence units (RFU) ±SD (n=3-4/group at day 3 and n=7-8/group at day seven), p=0.0006 Adh15-LO-1 vs. AdlacZ at day seven, Mann-Whitney test.*

Protein expression of the transduced human 15-LO-1 was detected in paraffin sections by immunohistochemistry. Expression was detectable in liver and spleen, but not in lungs, kidneys, or aorta. Figure 2D shows expression of the human 15-LO-1 in liver and spleen three days after the gene transfer. 15-LO-1 was expressed uniformly in liver, whereas in spleen, the expression was clearly localized to the perifollicular zone, the interface between the white pulp and the red pulp. No human 15-LO-1 protein was detected from the AdlacZ-transdued mice. At the seven-day timepoint, the expression was still visible, but not as strong as at the earlier timepoint (data not shown).

15-LO-1 induces lipid peroxidation and apoptosis. Lipid peroxidation was analysed in mouse livers, since these tissues showed the highest mRNA and protein expression. There were significantly more lipid peroxidation products MDA and 4-hydroxynonenal (4-HNE) produced in the livers of the Adh15-LO-1-transduced mice than in the AdlacZ-transduced mice at both time points, with a 3.5-fold increase at day three and 2.0-fold increase at day 7 (Figure 3A).

Apoptosis was detected in mouse livers by two different methods: immunohistochemical detection of the active form of caspase-3 from paraffin sections, and enzymatic activity of caspase-3 from tissue homogenates. An increased number of cells staining positively for the active form of caspase-3 was detected in the livers from Adh15-LO-1-transduced mice compared with those from AdlacZ-transduced mice (Figure 3B). The caspase-3 positive cells exhibited typical morphology of apoptotic cells, *i.e.* rounded shape, condensation of chromatin and cell shrinkage (Figure 3C). There was a significant 32-fold increase in caspase-3 enzymatic activity in the liver samples from Adh15-LO-1-transduced mice seven days after the gene transfer compared with the control samples from AdlacZ-transduced mice (Figure 3D). Three days after the gene transfer, caspase-3

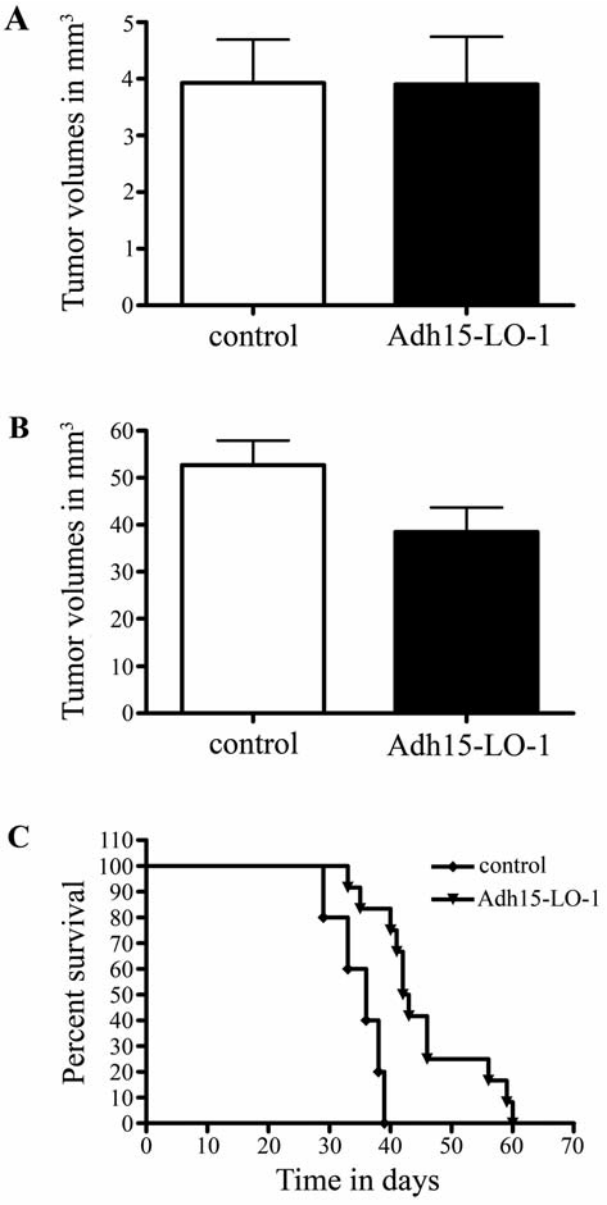

Figure 4. *Tumor volumes in Adh15-LO-1-treated and control animals two weeks (A) and four weeks (B) after BT4C cell implantation and gene therapy. C: Kaplan-Meier survival analysis of rats after Adh15-LO-1 gene therapy. After implantation of BT4C cells, rats were treated with Adh15-LO-1 (n=12) or followed up as control animals (n=5). The median survival of the treated and control groups were 42.5 days and 36.0 days, respectively (p=0.001).*

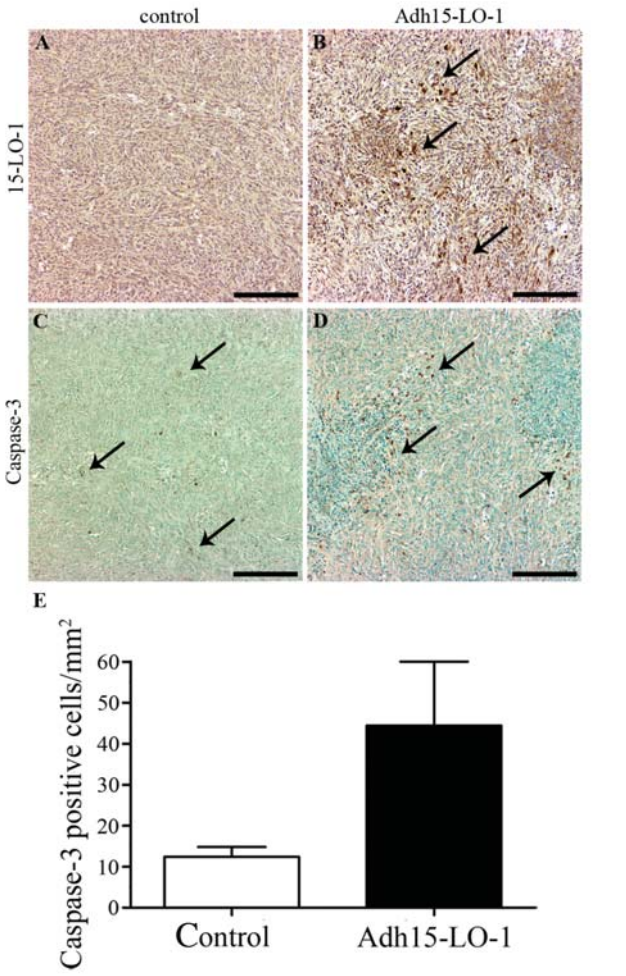

Figure 5. *Characterization of Adh15-LO-1 treatment effects in BT4C malignant glioma seven days after gene therapy. Immunohistochemical staining showing the expression of human 15-LO-1 (A, B) and caspase-3 (C, D) in controls (left) and Adh15-LO-1-treated animals (right), as indicated by arrows. Scale bar 200 μm. E: Quantitative analysis of mean caspase-3 positivity (cells/mm^2) ±SD, n=3, p=0.1.*

activity was below the background (assay buffer) in all samples from AdlacZ-1-transduced mice and in all but one from Adh15-LO-transduced mice.

15-LO-1 reduces tumor volume and extends survival in the rat glioma model. Effect of 15-LO-1 on tumor growth was studied in a syngeneic rat model of malignant glioma. Tumor volumes were visualised by MRI one day prior to gene transfer (Figure 4A). The tumor sizes at the beginning were comparable in both Adh15-LO-1-treated and control animals (3.903±0.8394 mm^3 and 3.925±0.7676 mm^3, respectively). The second MRI (Figure 4B), two weeks afterwards, showed no significant difference in the tumor size between the groups either, but a tendency for smaller tumors in the Adh15-LO-1 group was clearly visible (38.49±5.164 mm^3 *vs*. 52.71±5.201 mm^3). This indicates that the tumor growth was slowed, but not completely suppressed. Four weeks after gene transfer, at the time of the third MRI screening, 50% of the Adh15-LO-1 treated animals were still alive, while none of the control animals had survived up to this timepoint (data not shown). We observed a statistically significant prolongation of survival

upon Adh15-LO-1 administration (Figure 4C). The median survival time in Adh15-LO-1-treated animals was 42.5 days and it was 36.0 days in control animals. There was no notable body weight loss in the treated group after gene transfer nor any other health deterioration, indicating that there was no significant toxicity associated with the treatment. Clinical chemistry analysis (red blood cells, white blood cells, haemoglobin, platelets, alanine aminotransferase, aspartate aminotransferase, creatinine, bilirubin and urea) did not show any changes after gene therapy treatment (data not shown).

Protein expression of the transduced human 15-LO-1 was identified from brain paraffin sections by immunohistochemistry (Figure 5A and B). The effect of 15-LO-1 expression on apoptosis was assessed by detection of the active form of caspase-3 (Figure 5C and D). A tendency for an increased number of apoptotic cells was found in Adh15-LO-1-treated animals in comparison with the control group. However, the result was not significant (p=0.1; Figure 5E).

Discussion

One of the most fatal and refractory cancer types in humans is malignant glioma, out of which, the most common and aggressive subtype is glioblastoma multiforme (GBM). In spite of advances in cancer therapies, there is still no curative treatment available for patients with glioblastoma. The standard approach consists of surgical debulking of the tumor, followed by radiotherapy and chemotherapy. Clearly, there is an urgent need to develop alternative treatments for GBM.

15-LO-1 and its reaction products are involved in many pathological conditions, for example inflammation, atherogenesis and carcinogenesis. Apoptosis is a fundamental phenomenon taking place in all of these pathological conditions. However, controversial results about the role of 15-LO-1 and its products in the regulation of apoptosis and tumor growth have been presented (8, 27).

The rationale for the use of Adh15-LO-1 gene therapy for GBM relies on the idea that treatment strategies inducing apoptosis in glioma cells may offer promising new therapeutic approaches (28). We also believe that 15-LO-1 may target the glioma apoptotic pathway in an effective manner, since the expression of the activated form of caspase-3 has been shown to be a good prognostic indicator in cancer patients (29). To study the effect of 15-LO-1 on glioma, we chose the syngeneic BT4C model of malignant glioma in BDIX rats as it does not involve any significant host immune responses against tumor cells *per se*, and therefore mimics human GBM well (30). MRI was used to assess the tumor growth both before the treatment and throughout the study.

To our knowledge, there are no previous reports about the outcome of 15-LO-1 gene therapy in malignant glioma. In this study we demonstrated for the first time that 15-LO-1 has potential to significantly prolong survival in this model of malignant glioma. We found that the treatment group lived significantly longer than the control group. Additionally, it was evident that Adh15-LO-1 injection resulted in a delay in the tumor growth, although the results were not statistically significant. We would like to point out that in this study MRI was used to verify the presence and size of tumors before gene therapy. Therefore, we were able to visualise tumor volumes with respect to the applied treatment.

It is likely that antitumorigenic properties of 15-LO-1 in GBM are not solely due to its proapoptotic actions. We have recently shown that 15-LO-1 possesses strong antiangiogenic activity that is manifested by down-regulation of vascular endothelial growth factor receptor 2 (15). GBMs are among the most vascularised tumors in humans, which makes them a good target for antiangiogenic therapies (31). Furthermore, cells with increased expression of caspase-3 are more prone to radiation treatment (32), making 15-LO-1 a good candidate for future testing in combination therapy. Our results show that 15-LO-1 is a promising candidate for gene therapy of GBM.

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