Tumour-specific activation of the sodium/iodide symporter gene under control of the glucose transporter gene 1 promoter (GTI-1.3)

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Abstract. Targeted transfer of a functionally active sodium iodide symporter (NIS) into tumour cells may be used for radioiodine therapy of cancer. Therefore, we investigated radioiodine uptake in a hepatoma cell line in vitro and in vivo after transfer of the sodium iodide symporter (*hNIS*) gene under the control of a tumour-specific regulatory element, the promoter of the glucose transporter 1 gene (GTI-1.3). Employing a self-inactivating bicistronic retroviral vector for the transfer of the *hNIS* and the hygromycin resistance genes, rat Morris hepatoma (MH3924A) cells were infected with retroviral particles and *hNIS*-expressing cell lines were generated by hygromycin selection. $^{125}I^-$ uptake and efflux were determined in genetically modified and wild type hepatoma cells. In addition, the iodide distribution in rats bearing wild type and genetically modified hepatomas was monitored. *hNIS*-expressing MH3924A cell lines accumulated up to 30 times more iodide than wild type hepatoma cells, with a maximal iodide uptake after 30 min incubation time. Competition experiments in the presence of sodium perchlorate revealed a decrease in the iodide uptake (80–84% decrease). Moreover, ouabain led to a loss of accumulated I^- (81% decrease) whereas 4,4'-diisothiocyano-2,2'-disulphonic acid stilbene (DIDS) increased the I^- uptake into cells (87% increase). However, a rapid efflux of the radioactivity (70%) was observed 20 min after $^{125}I^-$-containing medium had been replaced by non-radioactive medium. Lithium had no significant effect on iodide efflux. In rats, the *hNIS*-expressing tumours accumulated 22 times more iodide than the contralateral wild type tumour. In accordance with the in vitro data, we also observed a rapid efflux of the radioactivity out of the tumour in vivo. Dosimetric calculations resulted in an absorbed dose of 85 mGy in the wild type tumour and 830 mGy in the *hNIS*-expressing tumour after administration of 18.5 MBq ^{131}I. In conclusion, transduction of the *hNIS* gene under the control of the GLUT1 promoter element induces iodide transport in Morris hepatoma cells in vitro and in vivo. However, for therapeutic application additional conditions need to be defined which inhibit the iodide efflux out of the tumour cells.

Keywords: Sodium iodide symporter – Glucose transporter promoter – Gene therapy

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Introduction

A well-described strategy for gene therapy of cancer is to render tumour cells susceptible to non-toxic chemotherapeutic agents using suicide genes. Besides the combination of gene transfer and chemotherapy, combination of gene transfer and radioactive isotopes is another promising avenue for the treatment of cancer. For each of these potential treatments to achieve clinical utility, the therapeutic gene must be delivered to tumour cells in vivo while avoiding undesired expression in normal tissues [1]. This can be achieved with a specific promoter directing the expression of a heterologous gene to the cells of interest.

It is known that tumour cells have a higher glucose metabolism compared with normal tissue cells [2], and tumour-related changes in the expression of glycolysis-associated genes have been reported by several investi-

gators. Especially the type 1 glucose transporter (*GLUT1*) gene has been described as one of the early genes which are activated after transformation of cells with oncogenes such as *src*, *ras* or *fps* [3, 4, 5, 6, 7, 8, 9, 10]. An increase in *GLUT1* mRNA was found 4–6 h after induction of the p21 c-H-ras oncoprotein, whereas morphologic changes occurred 72–96 h later [8, 11]. The facilitative glucose transporter GLUT1 has also been shown to be upregulated in human tumours [12, 13]. Besides the regulatory elements for vascular endothelial growth factor (VEGF) and most glycolytic enzymes, the GLUT1 promoter is a target of the hypoxia inducible factor 1α (HIF1α). HIF1α increases in hypoxic areas and during stimulation with vasoactive hormones or factors such as angiotensin II, thrombin and platelet-derived growth factor. As a consequence of increased HIF1α levels, VEGF, GLUT 1 and glycolytic enzymes are upregulated, especially in hypoxic areas of the tumors. Therefore, expression of the therapeutic gene in these problematic areas can be obtained with the GLUT1 promoter. These features make the regulatory element of the *GLUT1* gene a promising tool for tumour-specific gene therapy. In mice the GLUT1 promoter sequence was mapped at 1.3 kb to +137 bp [14, 15] and two enhancer elements have been characterised.

The characteristic ability of thyroid follicular cells to accumulate iodide enables benign thyroid diseases and differentiated thyroid carcinoma to be successfully treated with radioiodide therapy. The complex process of iodide trapping in the thyroid tissue is initiated by the active transport of iodide and sodium ions into follicular cells, which is mediated by the sodium-iodide symporter (hNIS). The energy-dependent transport, which proceeds against an electrochemical gradient, is coupled to the action of Na^+/K^+-ATPase and is stimulated by TSH [16, 17, 18, 19, 20].

Employing the rat Morris hepatoma cell line MH3924A as an in vitro/in vivo system, we investigated the effect of retroviral transfer of the *hNIS* gene under control of the promoter element of the *GLUT1* gene on iodide accumulation in transfected cells. This type of study combines the use of a tumour-specific promoter and a gene leading to the accumulation of a beta-emitting isotope in cancer cells. In this case trapping centres in the tumour could create a cross-firing of beta particles, resulting in destruction of both transduced and non-transduced tumour cells. We report here that the transduction of the *hNIS* coding sequence under control of a tumour-specific regulatory element is sufficient to induce increased iodide uptake which is associated with a considerable efflux and no significant effects of lithium on iodide trapping.
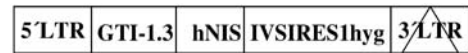

Fig. 1. Structure of the pSIR retroviral vector. pSIR, derived from Moloney murine leukaemia virus, is a self-inactivating retroviral vector which contains a deletion in the 3′LTR resulting in inactivation of the 5′LTR promoter. The vector contains GTI-1.3 as a regulating element for the expression of *hNIS* and the hygromycin resistance gene. A synthetic intron (*IVS*) is inserted to stabilise mRNA. To ensure simultaneous expression of the genes, an internal ribosomal entry site (*IRES*) from encephalomyocarditis virus (EMCV) is inserted

Materials and methods

Construction of a bicistronic retroviral vector for the transfer of the hNIS gene and the hygromycin gene. For transfer of the *hNIS* gene, a bicistronic retroviral vector based on the pSIR self-inactivating retroviral vector (Clontech, USA) was constructed. The *hNIS* gene and the hygromycin resistance gene were cloned downstream of the glucose transporter 1 promoter (GTI-1.3) taken from the GTI-1.3CAT vector (obtained from Murakami et al. [14]) (Fig. 1). To ensure simultaneous expression of the genes coding for the hNIS and for the hygromycin resistance and stability of the mRNA, a synthetic intron and an internal ribosomal entry site (IRES) from encephalomyocarditis virus (EMCV) were inserted between the genes. Synthetic intron, IRES and hygromycin resistance gene were excised from pIRES1hyg (Clontech, USA).

The self-inactivating vector contains a 176-bp deletion in the 3' LTR which removes enhancer sequences [21]. After reverse transcription, the 3' LTR is copied and replaces the 5' LTR, thereby inactivating the 5' LTR promoter and leaving the internal promoter (GTI-1.3) as the only promoter which drives the expression of downstream-located genes.

Cell culture, retroviral infection and generation of recombinant cell lines. The rat Morris hepatoma cell line MH3924A was cultured in RPMI1640 medium with glutamax-I supplemented with 20% fetal calf serum (FCS), and the transient packaging cell line BOSC23 [22], employed for the production of ecotropic retroviral particles, was cultured in RPMI1640 medium with glutamax-I supplemented with 10% FCS. All cells were supplemented with 100 IU/ml penicillin and 100 mg/l streptomycin (Invitrogen, Germany) and were cultured at 37°C, in an atmosphere of 95% air and 5% CO_2.

For transient packaging of the retroviral DNA containing the genes of interest and a hygromycin resistance gene (Fig. 1), a lipofection of BOSC23 cells was done. After 2 days the medium containing the retroviral particles was centrifuged to remove detached BOSC23 cells and used for the infection of the MH3924A cells in the presence of polybrene at 8 µg/ml overnight. To select for cells infected with retroviral particles containing the NaI symporter and hygromycin resistance gene under control of the GLUT1 promoter and stable integration of the virus DNA into the host genome, the cells were treated with 425 µg/ml hygromycin for 3 weeks until resistant cell lines were established.

Measurement and modulation of the 125I uptake and efflux. The iodide uptake was performed as described previously by Haberkorn et al. [23]. All experiments were done in triplicates and re-

peated at least twice. In the presence of 74 kBq Na^{125}I (Amersham, Germany; specific activity 625 MBq/µg; radioactive concentration 3.7 GBq/ml; radiochemical purity 99.3%), wild type MH3924A tumour cells and recombinant cell lines were incubated for 1 h. The cell counts were determined in a Coulter counter (Beckman Coulter, Germany). The cell viability was assessed by trypan blue staining. Cells treated with Na^{125}I were washed twice with ice-cold PBS and lysed with 0.3 M sodium hydroxide on ice. The radioactivity in both cell lysates and medium was measured using an automated NaI(Tl) well counter (Cobra II, Canberra Packard, USA).

To determine the iodide uptake in relationship to the incubation time, the recombinant cell line GTI-1.3hNIS43 and wild type MH3924A cells were cultured with 74 kBq Na^{125}I for 1, 2, 5, 10 or 30 min or 1, 2 or 4 h. Washing and counting was performed as described. For modulation of the iodide uptake, hNIS-expressing and wild type cells were incubated for 1 h in Na^{125}I medium (74 kBq) or Na^{125}I medium supplemented with 10 or 50 µM sodium perchlorate (Sigma), 100 or 300 µM of the anion channel blocker 4,4'-diisothiocyano-2,2'-disulphonic acid stilbene (DIDS; Sigma) or 500 µM ouabain (Sigma), an inhibitor of the Na$^+$/K$^+$-ATPase. Thereafter, the cells were washed, lysed and counted as described.

To determine the ^{125}I efflux, recombinant and wild type cells were incubated for 1 h with medium containing 74 kBq Na^{125}I. After the cells had been washed twice, three wells were lysed immediately. To the remaining wells was added fresh non-radioactive medium. The cells were again incubated for 2, 4, 6, 8, 10, 12, 16 or 20 min and immediately lysed as described. To investigate the effect of lithium on the ^{125}I efflux, cells were incubated for 24 h with 2, 10 or 50 mM lithium chloride (Merck, Germany). Thereafter, an efflux experiment was performed in the presence of LiCl$_2$ as described above. After incubation for 1 h in the presence of 74 kBq Na^{125}I, the radioactive medium was removed, and the cells were washed twice and incubated in non-radioactive lithium-containing medium for 10 or 20 min and lysed.

Measurement of the 131I uptake in tumour tissue of rats. The experiments were performed in compliance with the current version of the German Law on the Protection of Animals. GTI-1.3hNIS43 tumour cells (8×10^6) were transplanted subcutaneously into the right thigh or 4×10^6 wild type MH3924A cells were transplanted into the left thigh of 6-week-old male ACI rats weighting 200–250 g. For imaging studies, which were performed under general gaseous anaesthesia (40% O$_2$/ 60% N$_2$O/1% halothane), only animals bearing tumours with a minimum size of 10 mm in diameter were accepted. After injection of 14.8–18.5 MBq ^{131}I (corresponding to 481–601 MBq/m^2) in 200 µl of 0.9% NaCl into the lateral tail vein of the rats, scintigraphic images were taken using a 10-inch scintillation-camera (Searle-Siemens, Germany). The time-dependent relative accumulation of radioactivity in different regions of interest (ROIs), e.g. the heart, the liver, the tumour, the bladder and the whole animal, was monitored in four animals at 1, 2, 4, 6 and 24 h post injection. The absolute amount of radioactivity (% injected dose/g tissue) was determined in 20 animals which were sacrificed 1, 2, 4, 6 or 24 h post injection, by analysing the organs using an automated NaI(Tl) well counter (Cobra II, Canberra Packard, USA).
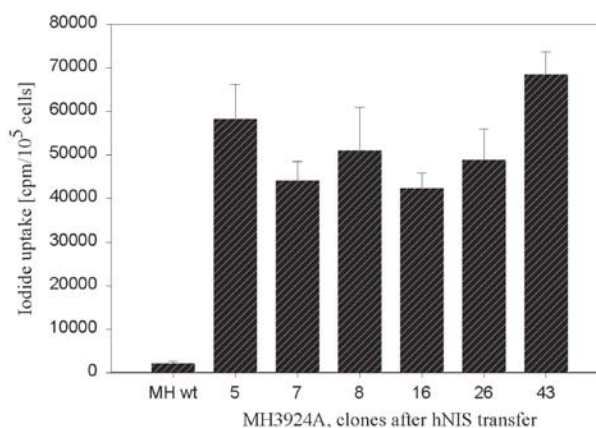

Fig. 2. Iodide uptake in MH wild type (wt) cells and MH cell lines that were stably transfected with *hNIS* under the control of GTI-1.3. The cells were incubated with ^{125}NaI for 1 h and the intracellular radioactivity was determined. Up to 30-fold higher iodide uptake was seen in MHGTI-1.3hNIS clones compared with MH wt cells. Data are mean values (*n*=3) ±SD

Results

Generation of hNIS-*expressing MH3924A cell lines*

After infection of MH3924A cells with recombinant retroviruses and selection with hygromycin, stable cell lines were established. In order to investigate the *hNIS* activity in the recombinant MH3924A cell lines, iodide uptake experiments were performed and the cell line GTI-1.3hNIS43 with the highest ^{125}NaI uptake was selected for further experiments. For the MH3924A and MHGTI-1.3hJTr43 cells we calculated an in vitro doubling time of 19 h and 18 h respectively. To exclude variations in iodide accumulation due to different cell volumes of wild type cells and transformed cells, the cell size was determined using a Coulter counter, revealing a diameter of 15.2 µm for MH3924A cells and 15.5 µm for MHGTI-1.3hJTr43 cells.

Na^{125}I *uptake and efflux in recombinant MH3924A cell lines*

After incubation with Na^{125}I for 1 h, up to 30-fold more iodide was transported into the *hNIS*-expressing hepatoma cell lines as compared with the wild type hepatoma cells (Fig. 2). The GTI-1.3hNIS43 cells presented the highest ^{125}I$^-$ uptake and, therefore, were employed for the following experiments. The iodide uptake was dependent on incubation time (Fig. 3), with a maximal ^{125}I$^-$ uptake after 30 min exposure. The radioactivity measured after 1 and 2 h ^{125}I$^-$ incubation was at a plateau level, implying a steady state.

Figure 4 presents the effect of DIDS, ouabain and sodium perchlorate on Na^{125}I uptake in the GTI-1.3hNIS43
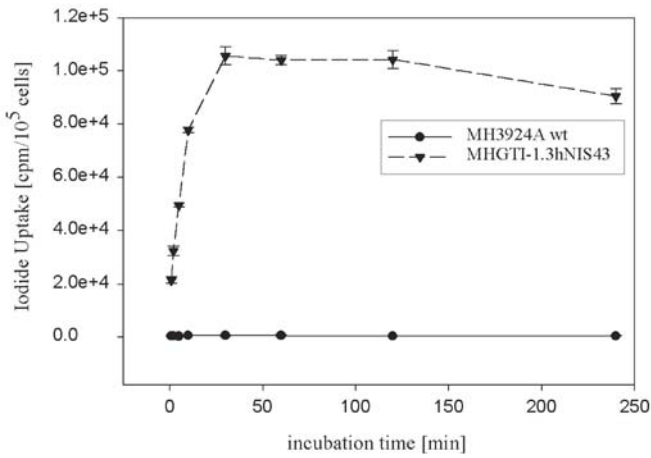
Fig. 3. Time course of iodide uptake by MH wild type (wt) and MHGTI-1.3hNIS43 cells. The cells were incubated with ^{125}NaI for 1, 2, 5, 10, 30, 60, 120 or 240 min and intracellular radioactivity was determined. Iodide uptake by MHGTI-1.3hNIS43 was rapid and became maximal within 30 min, whereas uptake by MH wt remained low. Data are mean values (*n*=3) ±SD
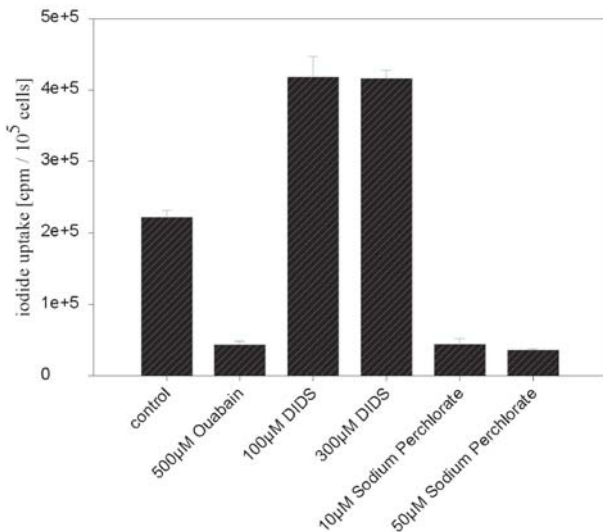

Fig. 4. Modulation of iodide uptake by sodium perchlorate, ouabain and DIDS. After 1 h incubation with ^{125}NaI, the uptake of iodide was decreased in the presence of ouabain (−81%) and sodium perchlorate (−84% at 50 μ*M*) and increased with DIDS (+87% at 100 μ*M*) compared with the untreated control. Data are mean values (*n*=3) ±SD

cell line. In the presence of 10 or 50 μ*M* sodium perchlorate we observed an inhibition of iodide accumulation in the GTI-1.3hNIS43 cells by 80% and 84% respectively. Addition of ouabain, which is an inhibitor of the Na$^+$/K$^+$-ATPase led to the loss of 81% of the accumulated I$^-$. The anion channel blocker DIDS had the opposite effect, as it increased the I$^-$ uptake into the cells by 87% with no difference at concentrations of 100 and 300 μ*M*.
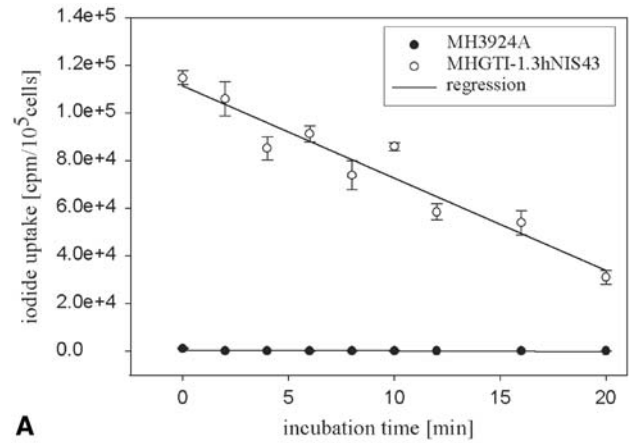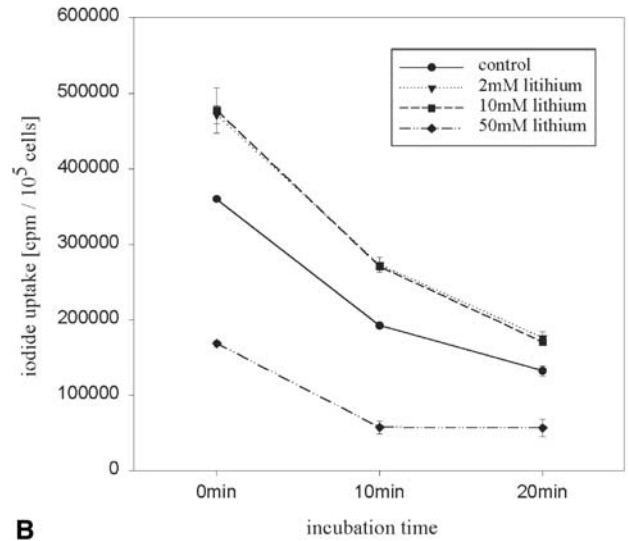

Fig. 5. A Iodide efflux from MHGTI-1.3hNIS43 cells after 1 h incubation with ^{125}NaI. Within 20 min 67% efflux was observed. **B** Cells were treated for 24 h with 2, 10 or 50 m*M* lithium and efflux was determined. Data are mean values (*n*=3) ±SD

To determine the isotope efflux, the iodide uptake was allowed to proceed for 1 h, at which time a steady state level of accumulation was achieved. After the medium had been replaced by non-radioactive medium, the amount of ^{125}I$^-$ present in the GTI-1.3hNIS43 cell lysates was determined as a function of time. The cellular radioactivity was constantly released into the medium and 67% efflux was observed after 20 min, indicating that the radiotracer was not trapped in the recombinant hepatoma cells (Fig. 5A). The addition of 2 and 10 m*M* lithium to the culture medium led to an increase in the initial ^{125}I$^-$ uptake. However, a 62% efflux during 20 min was observed. The addition of 50 m*M* lithium led to an inhibition of 50% in the initial iodide uptake compared to cells without lithium, with 70% efflux after 20 min (Fig. 5B).
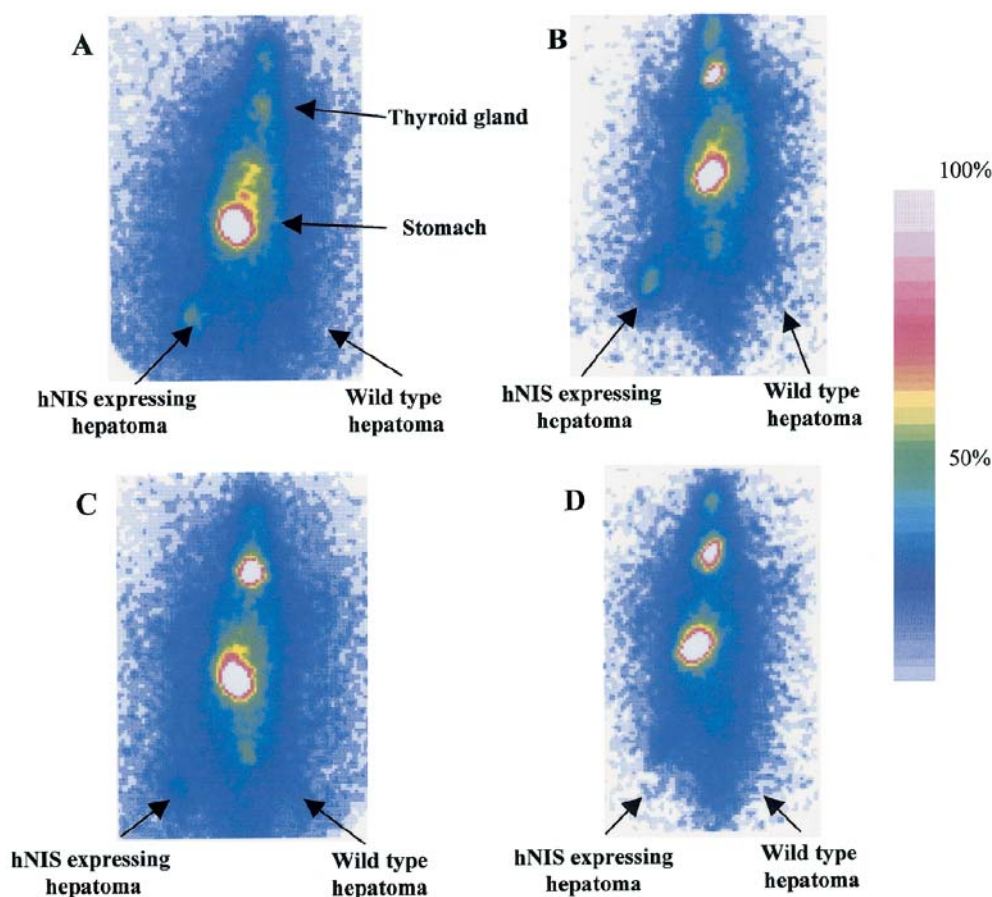
^{131}I uptake in Morris hepatoma in rats

Consistent with the data obtained from the in vitro studies the *hNIS*-expressing tumour tissue significantly accumulated $^{131}I⁻$, leading to scintigraphic visualization, whereas only low iodide uptake was observed in the wild type hepatoma (Fig. 6). In the genetically modified tumours the tracer accumulation increased to a maximum level during the first hour after administration, followed by a decrease in the intratumoral radioactivity, resulting in a biological $^{131}I⁻$ half-life in *hNIS*-expressing tumours of 14.1 h within the first 24 h after tracer administration. In contrast, the thyroid showed increasing tracer accumulation during the examination period (Fig. 7). The ex vivo quantitation of the $^{131}I⁻$ uptake (% injected dose/gram tissue) of the tumours and of various organs was evaluated 1, 2, 4, 6 and 24 h after tracer administration (Fig. 8). In the *hNIS*-expressing tumours, up to 23-fold higher iodide accumulation after 1 h was detected when compared with the wild type tumour, corresponding to 4.7% of the injected dose (ID)/g tissue. As a positive control, 23% ID/g could be achieved in the thyroid gland at 1 h after tracer administration. However, 2 h after tracer administration a decrease of radioactivity in the *hNIS*-expressing tumour occurred (4.2% ID/g) in contrast to the thyroid gland, where iodide is actively
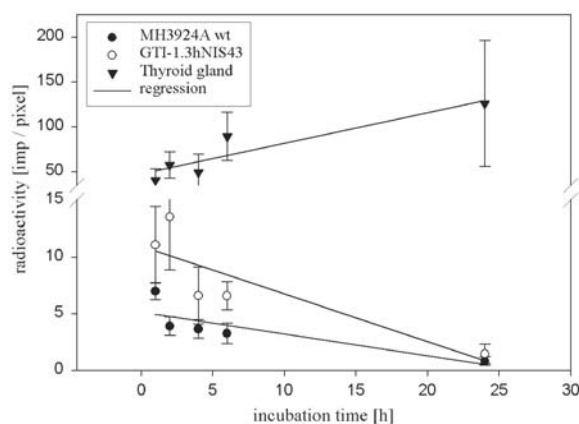

Fig. 7. $^{131}I⁻$ uptake in *hNIS*-expressing and wild type Morris hepatomas at 1, 2, 4, 6 and 24 h after radiotracer administration in rats [impulses (imp)/pixel]. A decrease in radioactivity occurred in the *hNIS*-expressing tumours within 24 h after administration, whereas an increase in tracer accumulation could be observed in the thyroid gland. Data are mean values (n=4) ±SD

organified, resulting in 32% ID/g tissue. At 24 h p.i. only a twofold accumulation (0.4% ID/g) was observed compared with the wild type tumour. Using MIRDOSE 3* [24] we calculated a cumulated activity of 2,879 MBq×s

Fig. 8. Organ distribution of the radiotracer at different times after tracer administration in tumour-bearing ACI rats [% ID (injected dose)/gram]. 4.7% ID/g was found in the *hNIS*-expressing tumours after 1 h (after 24 h: 0.4% ID/g). Data are mean values (*n*=4) ±SD
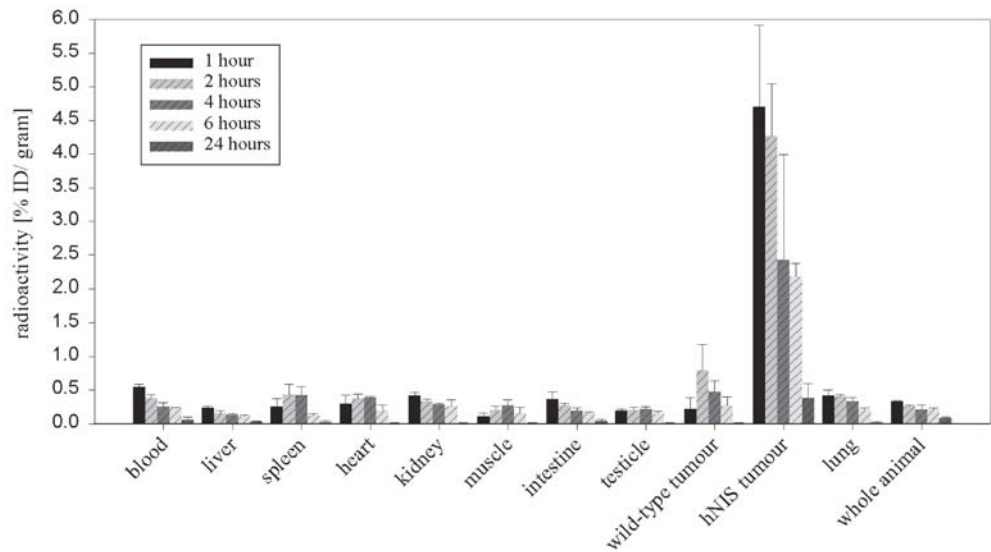
and 2.8×10^4 MBq×s for the wild type and the *hNIS*-expressing tumours, respectively. This activity resulted in an absorbed dose of 85 mGy (wild type tumour) and 830 mGy (*hNIS*-expressing tumour) after administration of 18.5 MBq ^{131}I.

Discussion

Thyroid cancer can be very effectively treated by radioiodine therapy. This is due to the expression of thyroidal genes such as thyroid peroxidase (*TPO*) and *NIS*, which lead to trapping of radioactive iodine in these tumours. Functioning thyroid cancer metastases can be detected and treated by administration of radioiodine, while avoiding adverse effects of ionising radiation to other organs, which do not concentrate radioiodine.

The cloning of the gene coding for the human sodium iodide symporter offered the possibility of *hNIS* gene transfer into non-thyroidal tumour cells, thereby inducing radioiodine uptake which may be used for imaging and treatment of non-thyroidal tumours with radioiodine [25]. Quantitation of iodide transport into genetically modified tissues may be done by dynamic positron emission tomography (PET) measurements of ^{121}I or ^{124}I uptake and pharmacokinetic models calculating transport rates. For scintigraphy with ^{99m}TcO$_4$ or ^{123}I, semiquantitative approaches using ratios of transfected to non-transfected tissues may be useful. Since radioiodine therapy of differentiated thyroid cancer has been proven to be very effective with no severe side-effects in the gastrointestinal tract or other tissues, transfer of the *hNIS* gene into non-thyroid tumours may be an attractive new therapeutic approach. However, to avoid adverse effects on the normal thyroid, this procedure makes sense only after thyroidectomy. Mandell et al. [26] demonstrated in vitro and in vivo iodide accumulation in several cancer cell lines, including melanoma, liver, colon carcinoma

and ovarian carcinoma cell lines, after transfection with the rat *NIS* gene. Stable cell lines [23, 27] and gene therapeutic approaches with tissue specific promoters [28, 29, 30, 31] were performed to direct the expression of the *NIS* gene to cells of interest.

The efficacy of radioiodine therapy of hepatoma after transfer of the *hNIS* gene could be enhanced using a tumour-specific promoter for the regulation of gene expression. The gene for the glucose transporter GLUT1 is known to be upregulated in most tumours [12, 13] and oncogene transformed cells [3, 4, 5, 6, 7, 8, 9, 10]. Therefore, the GLUT1 promoter should provide an efficient regulatory unit for tumour-specific gene expression. We could show in a series of experiments that under regulation of the GLUT1 promoter, reporter gene and *HSVtk* suicide gene expression was restricted to tumour cells and oncogene transformed cells [Sieger S et al., unpublished work]. In this study we investigated whether the transduction of the *hNIS* gene under the control of the GLUT1 promoter element is sufficient to induce iodide uptake in Morris hepatoma cells and tumours in rats. Employing a bicistronic self-inactivating retroviral vector for the *hNIS* and hygromycin resistance expression, we established MH3924A cell lines expressing the *hNIS* gene under the transcriptional control of the promoter region of the *GLUT1* gene (GTI-1.3). With respect to the wild type hepatoma cells, increased iodide uptake was observed in all recombinant cell lines. As the expression of viral DNA after its integration in the host genome can be affected by the chromatin structure of contiguous cellular sequences, the level of ^{125}I$^-$ accumulation varied significantly among individual cell lines. Additionally the balance of positive and negative acting host transcription factors determines the level of transcriptional activity of integrated viral DNA [32]. We observed an up to 30-fold higher radiotracer uptake when compared with the wild type cells, with a maximal accumulation after 30 min incubation (Fig. 3). In like man-

ner, Cos 7 cells transiently infected with the *hNIS* expression vector pcDNA3 accumulated tenfold more Na^{125}I than controls [25]. Mandell et al. [26] modified human melanoma, mouse colon carcinoma and human ovarian adenocarcinoma cells with a retroviral vector bearing the rat *NIS* gene and also observed an up to 35-fold increase in iodide uptake.

To evaluate whether the iodide accumulation was specifically induced by the *hNIS* activity, iodide uptake was determined in the GTI-1.3hNIS43 cells in the presence of sodium perchlorate, DIDS or the Na$^+$/K$^+$-ATPase inhibitor ouabain. Iodide uptake was decreased in the presence of ouabain as well as of sodium perchlorate, indicating that a functional *hNIS* is expressed in the genetically modified cell lines (Fig. 4). In contrast, the potent erythrocytes anion channel blocker DIDS, which has been described to stimulate the initial influx of iodide in the rat thyroid cell line FRTL5 [17] and in porcine thyroid cells [20], caused an 87% increase in uptake in the GTI-1.3hNIS43 cell line.

Non-thyroid, *hNIS*-expressing cells do not organify iodide, with the consequence that the concentration of intracellular iodide will drop proportionally to the external iodide concentration (Fig. 5A). Lithium was found to inhibit the release of iodine from the thyroid. Therefore, lithium was used to enhance the efficacy of radioiodine treatment of differentiated thyroid cancer [33, 34, 35]. In FRTL-5 rat thyroid cells and in primary cultures of porcine thyroid follicles, 2 m*M* lithium suppressed TSH-induced iodide uptake, iodide uptake stimulated by 8-bromo-cAMP, iodine organification and de novo thyroid hormone formation [35]. Koong et al. [36] described a lithium-induced prolongation (by up to 50%) of the biological and effective half-lives and increased accumulation of ^{131}I in metastases and thyroid remnants. A variety of actions have been described for lithium. These include inhibitory effects on the adenosine triphosphatase (ATPase) activity and on the inositol phospholipid metabolism with an impact on signal transduction, as well as alterations of cyclic adenosine monophosphate (cAMP), intracellular enzymes and in vitro responses of cultured cells to thyrotropin-releasing hormone. We performed efflux experiments with *hNIS*-expressing hepatoma cells in the presence of different concentrations of lithium. Lithium-treated cells revealed a higher initial iodide uptake compared with untreated cells when lithium concentrations of 2 and 10 m*M* were used, but still a 62% efflux was observed after 20 min. When lithium was used at a concentration of 50 m*M*, the initial iodide uptake was decreased by 50% in comparison to untreated cells, with a 70% efflux after 20 min (Fig. 5B). Our data showed only a marginal effect of lithium in hepatoma cells and cannot confirm the data obtained with thyroid cells. Therefore, it remains questionable whether the mechanisms described can be transferred to non-thyroid cells transfected with the *NIS* gene.

Another approach to achieve prolonged iodine retention in non-thyroidal tumours is simultaneous transfer of genes responsible for the iodide organification. Boland et al. [37] observed low levels of iodide organification in cells co-infected with both the *NIS* and the *TPO* gene in the presence of exogenous hydrogen peroxide. In a former study with rat hepatoma cells we could achieve high amounts of hTPO protein after retroviral transfer of the human *TPO* gene, but we were not able to measure TPO enzyme activity or enhanced accumulation of iodide [38]. Future approaches in our laboratory are directed towards stabilization of TPO by co-transfer of calreticulin and also the induction of thyroid-specific gene expression by transfer of thyroid specific transcription factors.

In vivo experiments with stable transduced MHGTI-1.3hNIS cells revealed maximal iodide uptake into the genetically modified hepatoma 1 h after the rats had been injected with ^{131}I. A decrease in iodide accumulation occurs as early as 2 h after tracer administration, whereas the thyroid gland accumulates ^{131}I until 24 h p.i. Although the hNIS protein favours iodide transport into the cells rather than efflux, the radioactivity continuously disappeared from the *hNIS*-expressing tumours and from different organs of the body except the thyroid (Figs. 6, 7 and 8). In a recent study Haberkorn et al. [23] transferred the *hNIS* gene with the EF1α regulatory element in rat Morris hepatoma cells and achieved an up to 235-fold higher iodide accumulation in vitro and a 17-fold higher iodide uptake 1 h after tracer administration in vivo. Using the GLUT1 promoter as a regulating element for the *hNIS* gene, we observed relatively less iodide accumulation with the GLUT1 promoter in vitro, but we achieved a higher (23-fold) iodide accumulation after 1 h in vivo. EF1α, the adult type elongation factor 1α, is one of the most abundant proteins in eukaryotic cells and therefore its promoter serves as a strong constitutive regulating element. The higher iodide accumulation achieved in vivo by using the GLUT1 regulatory element is evidence for the high activation of the GLUT1 promoter in the hepatoma model used in this study. However, different integration sites for exogenous genes in the cell lines may be responsible for differences in iodide uptake. An influence may also be expected from the different vector systems used: a retroviral vector with an intact long terminal repeat (LTR) and an internal promoter (EF1α) versus a self-inactivating retroviral vector with the GLUT1 promoter as the only active regulatory element. Furthermore, the copy number of integrated retroviral sequences may account for differences in activity. However, as we used the same stable cell line in vitro and in vivo with the same copy number in both types of studies, the observed higher uptake in vitro for the EF1α promoter but higher uptake in vivo for the GLUT1 promoter does not seem to be influenced by the copy number or the integration site. One possible interpretation may be the strong induction of the GLUT1 promoter in vivo by the transcription factor

HIF1α. HIF1α is upregulated especially in hypoxic areas, as well as in response to factors such as angiotensin II, thrombin and platelet-derived growth factor. Since tumours are highly heterogeneous with widely distributed hypoxic areas, we may expect induction of the GLUT1 regulatory elements by increased levels of HIF1α. Furthermore, the use of a self-inactivating retrovirus where the 5'LTR promoter is lost during reverse transcription leaves the GLUT1 promoter as the only active promoter in the construct, and no interference occurs between the 5'LTR and the internal promoter.

We determined a biological $^{131}I^-$ half-life in *hNIS*-expressing tumours of 14.1 h within the first 24 h after tracer administration. Compared with the amount of radioactivity in the blood, where we observed a biological $^{131}I^-$ half-life of 4 h, we achieved a prolonged accumulation of ^{131}I in the *hNIS*-expressing tumour. But still we expect the exposure time of genetically modified tumour cells to $^{131}I^-$ radiation to be too short for therapeutic relevance unless iodide is organified. From the accumulated activity of 2,879 MBq×s and $2.8×10^4$ MBq×s for the wild type and the *hNIS*-expressing tumours respectively, we calculated an absorbed dose of 85 mGy (wild type tumour) and 830 mGy (*hNIS*-expressing tumour) after administration of 18.5 MBq ^{131}I. Shimura et al. [39] also reported an achieved dose of 4 Gy after administration of 37 MBq ^{131}I, which was not sufficient to inhibit the growth of rat FRTL-Tc cells.

This study focussed on characterisation of cells, transfected with the Na^+/I^- symporter under control of a tumour-specific regulatory element in vitro and in vivo. The transduction of the *hNIS* gene induces high accumulation of iodide in non-I^--concentrating hepatoma cells, indicating a strong GLUT1 promoter-induced transcription of the *hNIS* gene in tumour cells. Furthermore, the GLUT1 promoter seems to be attractive because of the heterogeneous pattern of tumours, including the presence of hypoxic areas. In these areas GLUT1 may be strongly induced by the transcription factor HIF1α. However, since the iodide is not organified in the genetically modified cells, the high influx is followed by a rapid efflux in vitro and in vivo. Nevertheless, the approach of targeted radioiodide therapy with the help of a tumour-specific regulatory element can lead to accumulation of iodide in cancer tissue in vivo. However, several problems remain to be overcome: for a gene therapeutic application of the *NIS* gene under control of a tumour-specific promoter, the delivery mechanisms (viral vectors as gene shuttles) for efficient tumour cell transduction with the recombinant DNA for the *NIS* gene need to be improved, and ^{131}I must be retained in the target tissue sufficiently long to obtain therapeutically relevant radiation doses in the tumours. A further option to improve therapy outcome is the use of biologically more effective isotopes such as the high LET-emitter astatine-211 [40]. In addition to its therapeutic application, tumour-specific *NIS* gene expression could provide a diagnostic tool for imaging gene transfer in non-thyroidal tumours and their metastases using ^{99}Tc, ^{123}I or ^{121}I [41].

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