Presence of MHC-I in rat glioma cells expressing antisense IGF-I-Receptor RNA

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Abstract

The supposed immunogenic character of glioma cells transfected with antisense IGF-I-Receptor (IGF-I-R) expression vector was tested for the presence of MHC-I currently present in cells of IGF-I antisense type. C6 rat glioma cell line was comparatively transfected in vitro with IGF I antisense (pMT-Anti-IGF I) or IGF I Receptor antisense (pMT-Anti-IGF I R) expression vectors. The wild and transfected cells were examined for the presence of IGF-I and MHC-I molecules. Using RT PCR technique, the transfected "antisens" cells showed total inhibition of IGF-I. The both transfected cultures of IGF-I and of IGF-I -R type were positively stained for MHC-I. Moreover "antisense IGF-I-R" cells as compared to "IGF-I antisense" cells showed slightly higher expression of MHC-I. The transfected cells showed also the feature of apoptosis in 60% of cells. The immunogenicity of IGF-I-R antisense glioma cells is related to MHC-I presence; therefore both approaches of antisense IGF-I and of antisense IGF-I-R could be use in paralel for cellular therapy of glioblastoma.

Key words: glioma, IGF I, IGF-I Receptor, antisense, MHC-I.

Introduction

Different strategies of gene therapy for treatment of gliomas have been proposed comming from 1990s [1, 2, 3]. Our strategy treatement is based on antisense [4, 5] anti - IGF-I (insulin-like growth factor I) technology [1]. IGF-I is a 70-amino acid polypeptide involved in tissue growth and differentiation [6, 7, 8, 9].

Deregulated expression of growth factors and/or their receptors, and especially of IGF-I, is associated as well with growth as with pathology of different diseases, including tumors [9, 10, 11, 12]. IGF-I acts via specific IGF-I receptor and subsequent activation of a protein tyrosine phosphorylic signal transduction cascade, similar to that of insulin action [13, 14]. IGF-I, mediated by IGF-I receptor has been reported to stop the apoptosis pathway in a variety of cell lines [9, 15]. The action of IGF-I on cellular metabolism depends on binding proteins (IGFBP), which prolong the half life of this factor and modify its interaction with receptor [16, 17]. The block of IGF-I synthesis, induces apoptotic and immunogenic phenomenons [18].

IGF-I is expressed at high levels in some nervous system-derived tumours, e.g. glioblastoma [19, 20] and tumour cell lines like C6 and CNS-1 rat gliomas [21, 22, 23].

Rat C-6 glioma cells become down-regulated in production of IGF-I when transfected with vector expressing IGF-I antisense RNA or IGF-I triple-helix forming oligonucleotides. The transfected cells lose tumorigenicity and elicit tumor specific immunity which leads to cure of established tumors. The immune response was shown to be mediated mainly by T lymphocytes as indicated by an abundant infiltration of the CD8 T cell subset in tumor tissues [12, 24]. Here we examine the possibility that the suppression of intracellular IGF-I in rat glioma cells, using antisense IGF-I-Receptor approach, alters the cell phenotype and the production of MHC-I as it was previously observed in IGF-I antisense approach [25, 26], and that this process is also associated with increase in programmed cell death - apoptosis.

Material and methods

Cell culture. C6 rat glioma cell line [21], was used. Cells were cultured in DMEM+F12 (v/v) (GIBCO-BRL) supplemen-

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ted with 10 % FCS, 2mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin, at 37° C and 5 % CO_2. Hygromycin B (Boehringer Mannheim) at a concentration of 0,5 mg/ml was added 48 hours after transfection to select for transfected cell line. Then, the concentration of hygromycin B was changed to 2 mg/ml after one weak and maintained with each change of fresh medium during the next 3-4 month.

Plasmids

The episome based plasmids pMT-Anti IGF-I and pMT-Anti IGF-I-Receptor were constructed as previously described [1,3,27]. The vector pMT-EP, under the control of the metallothionein, MT-I, inducible promotor was used as its base. The casette contains the Epstein-Barr Virus origin of replication and the gene encoding nuclear antigen I which together drive extrachromosomal replication. Down stream of the insertion site is a poly A termination signal followed by the hygromycin B and ampilicin resistance genes. The vector pMT-EP with the lac-Z reporter gene, as insert was used in control experiments [28].

Transfection

Cultures of cells, 60-80 % confluent, were transfected in 6-well plates utilizing a ratio of 1 µg plasmid DNA per 400 000 cells. The FuGENE 6 Transfection Reagent (Boehringer Mannheim) was used according to the supplier's instructions. To determine the efficiency of transfection, the process was carried out using the pMT-EP construct containing lac-Z as a reporter gene. Cell cultures were washed in PBS and incubated at 37° C in the presence of the staining solution which contained 5mM $K_3Fe(CN)_6$, 2mM $MgCl_2$, 0,8 mg/ml X-gal made in PBS.

The cell lines, transfected and non transfected were comparativly analyzed for expression of IGF-I, IGFI-R and MHC-I in the presence and absence of ZnSO4 (stimulation of IGF-I metallothionin promoter).

RT PCR (Reverse transcriptase - polymerase chain reaction)

RNA from cells was isolated using High Pure RNA Isolation Kit (Roche Diagnostics GmbH nr 1828665), and the obtained mRNA was verified in 1,5% agarose gel stained with ethidium bromide. The applied components of RT PCR were used according Reverse Transcription System Promega Corporation (nr A3500). Samples composed as mentioned in Table 1. were amplified for 30 cycles.

Each cycle consisted of the following steps: denaturation at 94°C for 30 sec, then at 65°C for 30 sec.; annealing for 60 sec, primer extension at 72°C for 5 min in a DNA thermal cycler (GeneAmp PCR System 2700, Applied Biosystem).

The following primers were used for RT PCR study of:
IGF-I:
Primer "a"
Forward: TAG TCC CTG CCT CTT AAG AG
Reverse: AGG GGC GTT AAA ACT TGG GT
(sequence according "rgd" Rat Genome Database; primers synthetized in Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw).

Table 1.

Components	Sample (µl)
cDNA	2
dNTPs, 10mM	1,8
$MgCl_2$, 25mM	7,5
RT 10x Bufor	9,8
rev IGF-I primer, 20µM	2
dir IGF-I primer, 20µM	2
DNA Pfu Polimerase (2,5)	0,5
Nuclease free water (100µl)	74.4

IGF-I:
Primer "b"
Forward: TCT GCT GAA GCC TAC AAA GT
Reverse: TTC CTC AAG CAG CAA AGG AT

IGF-I-Receptor:
Primer"c"
Forward: GAC AGT GAA TGA GGC TGC AA
Reverse: TCT CCA CCT CTG GCC TTA GA

MHC-I:
Primer "d"
Forward: ACA CTC GCT GCG GTA TTT CT
Reverse: CCT TGG CTT TCT GTG TCT CC

(primers synthetized in Institute of Pharmacology, Polish Academy of Sciences, Cracow)

Amplification products were analyzed in 1,5 % agarose gel; each well was loaded by 0,5 ug DNA stained by ethidium bromide. Standard markers were used (PCR markers, Promega Corporation, nr G316A).

Results

Morphological characterisation of tumoral cells.

C6 glioma cells demonstrated morphological characteristics similar to those previously described [1] (Fig. 1). The percent of IGF-I positive cells ranged from 70-90%. Each cell line was was subcloned to obtain IGF-I positive clones. IGF-I antisense transfected cells (pMT-Anti IGF-I) as well as IGF-I-R antisense transfected cells (pMT-Anti IGF-I-R) did not stain immunocytochemically for IGF-I [1, 27]. The frequency of transfection for the pMT-EP- LacZ vector, as determined by staining with X-gal at 24 hours after transfection, was in between 3-10 % for glioma lines (Fig. 2). In cultures of both IGF-I antisense and IGF-I-R transfected cells, approximately 40-50 % of the cells became rounded and detached. These cells were all apoptotic. In contrast, evidence of apoptosis was observed in less than 5-10 % of non-transfected cells. The apoptotic changes occurred within 5-6 hours after incubation of transfected cells in the presence of 60 microM of $ZnS0_4$. These results were confirmed in six separate experiments ($P < 0, 01$).

Figure 1. In vitro culture of C6 glioma cells. The cloned cells showing oftenly elongated shape, present a pseudo-fibroblastic character. The apoptosis phenomenon, oval cells with vacuolization is rare (less than 5%). X400

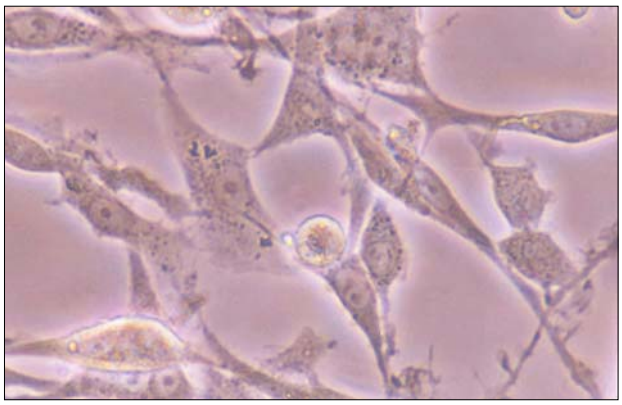
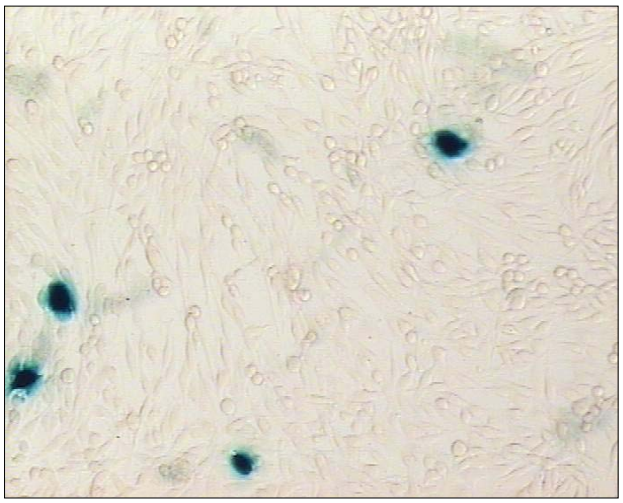


Figure 2. In vitro C6 glioma cells transfected with "antisense" pMT-Anti IGF-I-R vector and with pMT-EP construct containing lac-Z as a reporter gene. The transfected cells are morphologocally different from non transfected cells; they are all elongated. Note blue-green cells (X-gal staining) of cells transfected with beta-galactosidase expressing vector (lac-Z). X400



Molecular characterization of tumoral cells.

Analysis of C6 rat glioma, parental, non transfected cells is shown in the RT PCR gels (Figs. 3, 4a and b). Using primer for IGF-I and IGF-I-R, the presense of these two genes was demonstrated in non transfected cells. The DNA of non-transfected cells is distributed in 300-400 bp (Fig. 3) and 200 bp bands (Figs. 4a and b) corresponding to the different primers.

As far as transfected cells are considered, the presence of MHC-I was confirmed in "antisense IGF-I cells" [12] using RT PCR technique. These cells showing absence of IGF-I, demonstrated the expression of MHC-I after stimulation with Zn SO4 (Figs. 5a and b). The same striking phenomenon was observed in "antisense IGF-I-Receptor" cells, also stimulated with ZnSO4 (Figs. 6a and b), indicating the presense of MHC-I. Non stimulated cells show very weak bands of 200 bp or their absence using MHC-I primers.

Figure 3. The example of RNA isolation and of amplified DNA in C6 non transfected, parental cells. M - marker (PCR Markers, Promega Corporation, nr G316A. 1, 2, - isolated samples of RNA. 3, 4, 5, 6, 7 - amplified samples of DNA: note 300-400 bp bands confirm the expression of IGF-I using IGF-I primer "a" (see Material and methods).

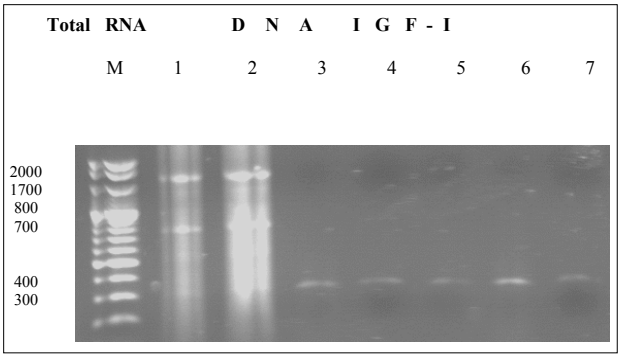


Figure 4a. C6 parental cells. The expression of IGF-I demonstrated by RT PCR. M- marker; 1,2,3 - 200 bp bands of amplified DNA using IGF-I primer "b" (see Material and methods).

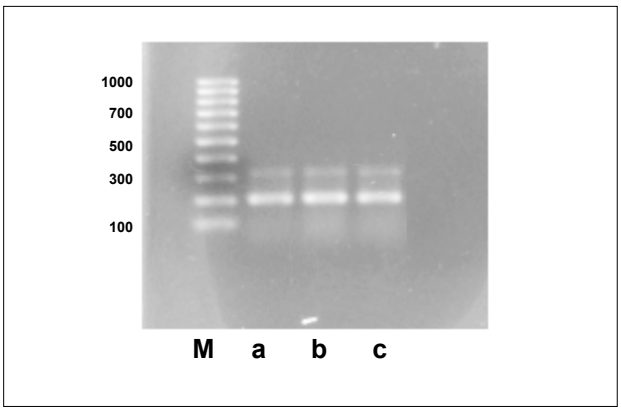
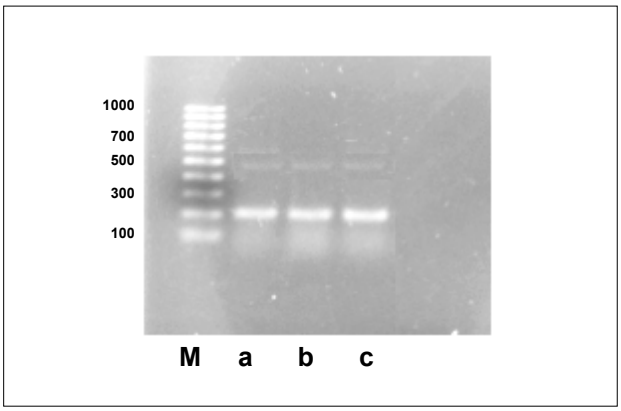


Figure 4b. C6 parental cells. The expression of IGF-I-Receptor demonstrated by RT PCR. M- marker; 1,2,3 - 200 bp bands of amplified DNA using IGF-I-R primer "c"; 300 bp is non specific (see Material and methods).



Discussion

Other studies have already shown, that the antisense strategy directed to the receptor of IGF induces a programmed cell death of tumor cells [9, 18, 22, 29]. As antitumor IGF-I strate-

Figure 5a. C6 glioma cells transfected with pMT-Anti IGF-I. Absence of expression of IGF-I demonstrated by RT PCR. M- marker; a, b, c, d, e, f - amplified DNA using IGF-I primer "b" (see Material and methods). The lines a, b, c correspond to non stimulated cells, and the lines d, e, f correspond to cells stimulated with Zn SO4.

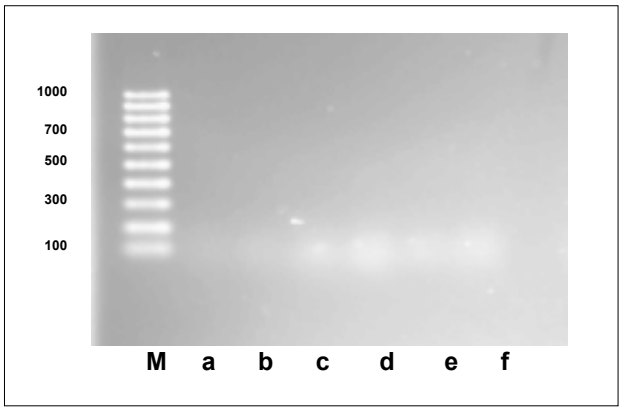


Figure 5b. C6 glioma cells transfected with pMT-Anti IGF-I. M- marker; a, b, c, d, e, f - amplified DNA using MHC-I primer "d" (see Material and methods). The lines a, b, c correspond to non stimulated cells, and the lines d, e, f correspond to cells stimulated with Zn SO4. Note the expression of MHC-I demonstrated by RT PCR in cells corresponding to lines d, e, and f.

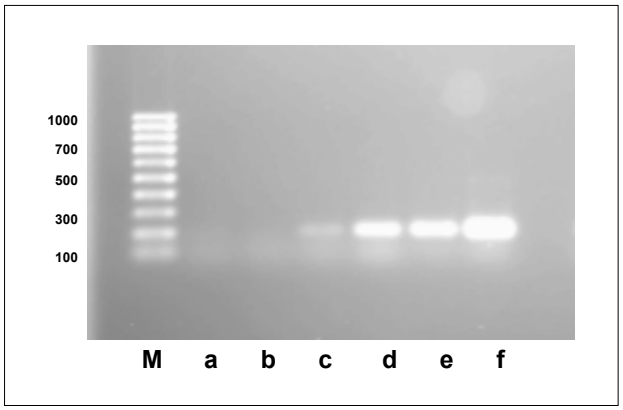


Figure 6a. C6 glioma cells transfected with pMT-Anti IGF-I-Receptor. Absence of expression of IGF-I demonstrated by RT PCR. M- marker; a, b, c, d, e, f - amplified DNA using IGF-I primer "b" (see Material and methods). The lines a, b, c correspond to non stimulated cells, and the lines d, e, f correspond to cells stimulated with Zn SO4.

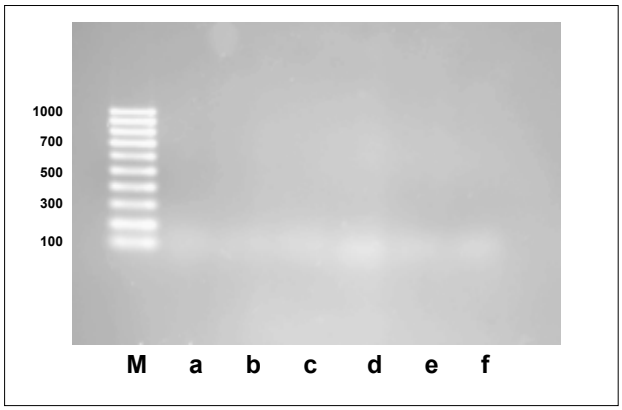
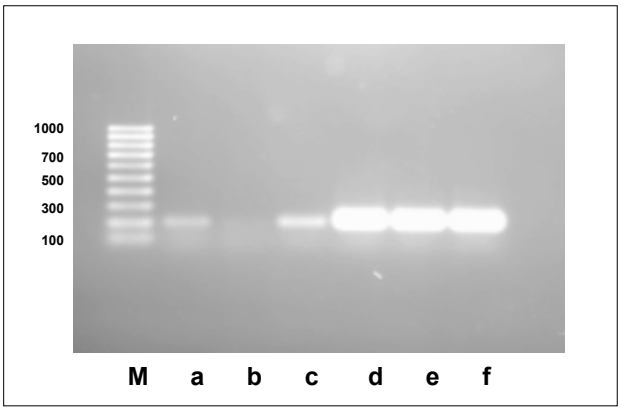


Figure 6b. C6 glioma cells transfected with pMT-Anti IGF-I-Receptor. M- marker; a, b, c, d, e, f - amplified DNA using MHC-I primer "d" (see Material and methods). The lines a, b, c correspond to non stimulated cells, and the lines d, e, f correspond to cells stimulated with Zn SO4. Note the expression of MHC-I demonstrated by RT PCR especially in cells corresponding to lines d, e, and f and weakly in non stimulated cells (c).



gy for the neuroectodermal neoplasia - glioblastoma multiforme - have evolved, we have showed that a relationship may exist between the anti-tumor immune response and apoptosis. We demonstrated that IGF-I antisense transfection induce MHC-I molecules, and that using vectors encoding MHC-I antisense cDNA: 1) MHC-I expression, related to IGF-I antisense and triple helix transfection, can be reversible; 2) suppression of MHC-I expression in immunogenic IGF-I antisense transfected cells is following by the strike decrease of apoptotic cells [30]. These data define the immune and apoptotic characteristics of tumoral cells selected for cellular therapy of glioma, and permited introduce the IGF-I antisense and triple-helix technology as a new gene therapy approach for treatement of human glioblastoma [23].

In here presented work down regulation of cellular IGF-I mRNA is accompanied by a decrease of cellular IGF-I. Transcription of the IGF-I gene is down regulated for 4 - 6 passages in rat glioma cells transfected with pMT-Anti IGF I-R vector. In control experiments using the same vector carrying the lacZ gene in place of IGF-I antisense DNA sequence, we have showed 24 hours after transfection a 3-10% transgene frequency (100 % after three weeks) and strong cytoplasmic staining for X-gal in rat C-6 cells. The induction of IGF-I antisense RNA in transfected cells was followed by change in cell morphology, increase in apoptosis and enhanced expression of MHC-I. Transfected cells were long and narrow in shape and frequently string-like in appearance. This change was associated with down regulation in IGF-I protein. The change might be the signal either a reversion of the malignant phenotype or the recovery of some antigenic potential of these cells. As to the MHC-I expression, down-regulation of MHC-I due to action of IGF-I has been reported for experiments with rat thyroid cells [31]. This would be in agreement with results reported here concerning the inverse correlation between IGF-I and MHC-I protein expression in glioma cells. In tumor cells, the absence of IGF-I, when induced by IGF-I antisense technology, is associated with

massive apoptosis as previously demonstrated in transfected hepatoma cells [32]. In here presented work, the occurrance of morphological features vacuolization of cytoplasm suggests that the intracellular growth factor may be a control point for modulating to apoptotic process of glioma cells. IGF-I has been reported to block the apoptosis pathway in a variety of cell lines [33]. In contrast rats given injections of C6 glioma cells expressing antisense IGF-I-R did not develop tumors and were protected from a subsequent challenge with wild-type C6 glioma cells for at least months [3]. However, total eradication of pre-existing wild type tumors was not described [3]. On the other side, a qualitative relationship between the level of IGF-I receptor and tumorigenesis in nude mice, which correlates to the extent of apoptosis has been shown [34]. When the function of IGF-I receptor is decreased, glioma cells undergo massive apoptosis. It was concluded for the IGF-I-R result, that this receptor activated by its ligand plays a protective role against programmed cell death. This protection was even more striking in vivo than in vitro [34]. Another possible interpretation could be that an immune response occurring in the animals inhibits tumorigenesis. This is probably because nude mice do have a residual immune system containing both natural killer cells and B lymphocytes [35]. Progress in defining the pathophysiological role of signalling and downstream of IGF-I-R in neoplasia might lead to the development of novel targeting strategies [36]. Recent research has shown that targeting IGF-I-R leads to impressive antineoplastic activity in many in vitro and in vivo models of common human cancers. Strategies that have been used include [36]: administration of molecules to interfere with ligand binding to IGF-I-R, such as IGFBPs, peptide or small-molecule competitive binding antagonists, or blocking anti-receptor antibodies [3, 37, 38], antisense or small interferring RNA strategies to reduce receptor expression [40]; introduction of a dominantnegative IGF-I-R to interfere with receptor action use of small-molecule IGF-I-R-specific tyrosine kinase inhibitors [41]; and targeting signalling pathways downstream of IGF-I-R with agents such as AKT or TOR inhibitors [42, 43]. The hypothesis that targeting the IGF-I-R will increase the efficacy of other antineoplastic treatments is based on evidence that survival signals originating at this receptor limit the efficacy of other treatments designed to induce apoptosis [36].

The results presented in this study show that inhibition of IGF-I-R up-regulates MHC-I expression in transfected " antisense " glioma cells. We need to add that increased expression of protease nexin I which may reduce the tumorigenic potential of the C-6 glioma cells was also observed when the IGF-I "triple-helix" cells or IGF-I receptor "triple-helix" cells were injected into nude mice [24, 27]. On the other hand, in our work concerning mouse hepatoma cells transfected by IGF-I triple-helix approach [28], we have observed the decrease of cytokines like Il-10, which is a strong immunosuppressor, and TNF-alpha, which can act as a factor stimulating a tumoral growth. Moreover we have found the increased level of TAP 1 and 2 in these cells. A further elucidation of the relationship between the immune process, related to MHC-I or HLA system [44], and the apoptotic process is under investigation. Our antisense IGF-I aproach together with that of antisense IGF-I-R and other actually studied antisense strategies of gene therapies targeting bcl-2 [45], telomerase [46], urokinase-type plasminogen activator receptor (uPAR) [47], neuroglia related Cell Adhesion Molecule [48] or matrix metalloproteinases [49] constitute the begining of successfull clinical trial to win "glioblastoma battle".

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References

1. Trojan J, Blossey BK, Johnson T, Rudin S, Tykocinski M, Ilan Ju, Ilan J. Loss of tumorogenicity of rat glioblastoma directed by episome-based antisense cDNA transcription of insulin-like growth factor I. Proc Natl Acad Sci USA, 1992; 89: 4874-8.

2. Culver K W, Rarn Z, Wallbridge S, Ram Z., Walltridge S, Ishii H, Oldfield EH, Blaese RM. In vivo gene transfer with retroviral vector-producer cells for treatment of experimental brain tumors. Science, 1992; 256: 1550-2.

3. Resnicoff M, Sell C, Rubini M, Coppola D, Ambrose D, Baserga R. Rat glioblastoma cells expressing an anti-sense RNA to the insulin-like growth factor I (IGF-I) receptor are non tumorigenic and induce regression of wild-type tumors. Cancer Res, 1994; 54: 2218-22.

4. Izant JG, Weintraub H. Constitutive and conditional suppression of exogenous and endogenous genes by antisense RNA. Science, 1985 ; 229: 345-52.

5. Green P J, Pines O, Inouye M. The role of antisense RNA in gene regulation. Ann Rev Biochem, 1986; 55: 569-97.

6. Daughaday WH, Hall K, Raben MS, Salmon WD, Van den Brande JL, Wyk JI. Somatomedin: proposed designation for sulphation factor. Nature, 1972; 235: 107.

7. Froesch CS, Schwander J, Zapf J. Actions of insulin-like growth factors. Ann Rev Physiol 1985; 47: 443-67.

8. Han VKM, Hill DJ. In The Insulin-like growth factors : Structure and biological functions, Shofield PN (ed)., Oxford University Press, 1992; 178-219.

9. Baserga R. Oncogenes and strategy of growth factors. Cell 1994; 79: 927-30.

10. Rubin R, Baserga R. Biology of disease. Insulin-like growth factor I receptor. Its role in cell proliferation, apoptosis and tumorigenicity. Lab Invest, 1995; 73: 311-31.

11. Heldin CH, Westermark B. Growth factors as transforming proteins. Eur J Biochem, 1989: 184: 487-96.

12. Trojan J, Johnson TR, Rudin SD, Ilan Ju, Tykocinski ML, Ilan J. Treatment and prevention of rat glioblastoma by immunogenic C6 cells expressing antisense insulin?like growth factor I RNA. Science, 1993; 259: 94-7.

13. Werner H, Le Roith D. New concepts in regulation and function of of the insulin-like growth factors: implications

for understanding normal growth and neoplasia. Cell Mol Life Sci, 2000; 57: 932-42.

14. Adams TE, Epa VC, Garrett TP, Ward CV. Structure and function of the type I insulin-like growth factor receptor. Cell Mol Life Sci, 2000; 57: 1050-93.

15. Rodriguez-Tarduchy G, Collins MKL, Garcia I, Lopez-Rivas A. Insulin-like growth factor-I inhibits apoptosis in IL-3-dependent hemopoietic cells. Eur J Immunol, 1992; 149: 535-40.

16. Hva V, Oh Y, Rosenfeld RG. The insulin-like growth factor-binding protein superfamily. Endocr Rev, 1999; 20 : 761-87.

17. Zumkeller W, Westphal M. The IGF/IGFBP system in CNS malignancy. J Clin Pathol (Molec Pathol), 2001; 54: 227-9.

18. Upegui-Gonzalez LC, Duc HT, Buisson Y, Arborio M, Lafarge-Frayssinet C, Jasmin C, Guo Y, Trojan J. Use of the IGF-I antisense strategy in the treatment of the hepatocarcinoma. Adv Exp Med Biol, 1998; 451: 35-42.

19. Sandberg AC, Engberg C, Lake M, von Holst H, Sara VR. The expression of insulin-like growth factor I and insulin-like growth factor II genes in the human fetal and adult brain and in glioma. Neurosci Lett, 1988; 93: 114-9.

20. Antoniades HN, Galanopoulis T, Nevile-Golden J, Maxwell M. Expression of insulin like growth factor I and II and their receptor mRNAs in primary human astrocytomas and meningiomas : In vivo studies using in situ hybridization and immunocytochemistry. Int J Cancer, 1992; 50: 215-22.

21. Kiess W, Lee L, Graham DE, Greenstein L, Tseng L Y, Rechler M M, Nissley S P. Rat C6 glial cells synthesize insulin-like growth factor I (IGF-I) and express IGF-I receptors and IGF-ll / mannose 6-phosphate receptors. Endocrinology, 1989; 124: 1727-36.

22. Baserga R, Sell C, Porcu P, Rubini M. The role of IGF-I receptor in the growth and transformation of mammalian cells. Cell Prolif, 1994; 27: 63-78.

23. Trojan LA, Kopinski P, Mazurek A, Ly A, Jarocki P, Shevelev A, Trzos R, Pan Y, Gitis DJ, Niklinski J, Bierwagen M, Czapiewska LJ, Wei M, Chyczewski L, Michalkiewicz J, Henin D, Popiela T, Evrard P, Kasprzak H, Anthony D, Trojan J. IGF I triple helix gene therapy of rat and human gliomas. Roc Acad Med Bial (Ann Proc Med Sci), 2003; 48: 18-27.

24. Shevelev A, Burfeind P, Schulze E, Rininsland F, Johnson T, Trojan J, Chernicky C, Hélene C, Ilan J, Ilan J. Potential triple helix mediated inhibition of IGF-I gene expression significantly reduces tumorigenicity of glioblastoma in an animal model. Cancer Gene Therapy 1997; 4: 105-12.

25. Trojan J, Duc H, Upegui-Gonzalez L, Hor F, Guo Y, Anthony D, Ilan J. Presence of MHC-I and B-7 molecules in rat and human glioma cells expressing antisense lGF-1 mRNA. Neurosci Lett, 1996; 212: 9-12.

26. Anthony D, Pan Y, Wu S, Shen F, Guo Y. Ex vivo and in vivo IGF-I antisense RNA strategies for treatment of cancer in humans. Adv Exp Med Biol 1998; 451: 27-34.

27. Rininsland F, Johnson T, Chernicky C, Schulze E, Burfeind P, Ilan J. Suppression of insulin like growth factor type-I receptor by a triple-helix strategy inhibits IGF-I transcription and tumorigenic potential of rat C6 glioblastoma cells. Proc Natl Acad Sci USA 1997; 94: 5854-9.

28. Upegui-Gonzalez LC, Ly A, Sierzega M, Jarocki P, Trojan L A, Duc HT, Pan Y, Shevelev A, Henin D, Anthony D, Nowak W, Popiela T, Trojan J. IGF-I triple helix strategy in hepatoma treatement. Hepato/Gastroentero 2001; 48 : 660-6.

29. Baserga R. The insulin-like growth factor I receptor: a key to tumor growth? Cancer Res, 1995; 55: 249-52.

30. Ly A, Duc HT, Kalamarides M, Trojan LA, Pan Y, Shevelev A, François JC, Noël T, Kane A, Henin D, Anthony D, Trojan J. Human glioma cells transformed by IGF-I triple-helix technology show immune and apoptotic characteristics determining cell selection for gene therapy of glioblastoma. J Clin Pathol (Molec Pathol), 2001; 54: 230-9.

31. Saji M, Moriarty J, BanT, Singer D, Kohn L. Major Histocompatibility Complex class I gene expression in rat thyroid cells is regulated by hormones, methimazole and iodide as well as interferon. J Clin Endocrinol Metab, 1992; 75: 871-8.

32. Ellouk-Achard S, Djenabi S, De Oliveira GA, Dessay G, Duc HT, Zoar M, Trojan J, Claude JR Sarasin A, Lafarge-Frayssinet. Induction of apoptosis in rat hepatoma cells by expression of IGF-I antisense cDNA. J Hepatology, 1998; 29: 807-18.

33. Rodriguez-Tarduchy G, Collins MKL, Garcia I,. Lopez-Rivas A. Insulin-like growth factor-I inhibits apoptosis in IL-3-dependent hemopoietic cells. Eur J Immunol, 1992; 149: 535-40.

34. Resnicoff, M., Abraham, D., Yutanawiboonchai, W., Rotman, H.L., Kajustora, J. Rubin R. The insulin-like growth factor I receptor protects tumor cells from apoptosis in vitro. Cancer Res, 1995; 55: 2463-9.

35. Taghian A, Bucach W, Zietman A, Freeman J, Gioioso D, Smit HD. Quantitative comparison between the transplantability of human and murine tumors into the brain of NCr/Scd- nu/nu Nude and severe combined immunodeficient mice. Cancer Res, l993; 53: 5018-21.

36. Pollak MN, Shernhammer E,S, Hankinson SE. Insulin like growth factors and neoplasia. Nature Rev Cancer, 2004; 4: 505-18.

37. Maloney EK. Anti-insulin like growth factor-I receptor antibody that is a potent inhibitor of cancer cell proliferation. Cancer Res, 2003; 63; 5073-83.

38. Burtn D. A fully human monoclonal antibody to the insulin-like growth fades-I receptor blocks ligarid-dependent signaling and inhibits human tumor growth in vivo. Cancer Res, 2003; 63: 8912-21.

39. Jackson-Booth PG, Teny C, Lackey B, Lopaczynska M, Nissley P. Inhibition of the biological response to IGF-I in MCF 7 breast cancer cells by a new monoclonal antibody to the IGF-I-R. The importance of receptor down regulation. Hom Metab Res, 2003; 35: 850-6.

40. Bohula EA. The efficacy of small interfering RNA's targeted to the type I insulin-like growth factor receptor (IGF 11:3) is infIuenced by secondary structure in the IGF1 R transcript. J Biol Cheer, 2003; 278: 15991-7.

41. Garcia-Echeverria C. In vivo aiti-turrior activay of NVP-AEW541- A novel, potent and selective inhibitor of the IGF-IR kinase. Cancer Cell, 2004;5: 231-9.

42. Houghton P, Huang S. mTOR as target for cancer therapy. Curr Top Microbiol Immunol, 2004; 279: 339-59.

43. Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. Nature Rev Cancer, 2004; 4: 335-48.

44. Matthew L, Saiter B, Bhardwag N. Dendritic cells acquire antigen from apoptotic cells and induce class I restricted CTL. Nature, 1998; 392: 86-9.

45. Zhu CJ, Li YB, Wong MC. Expression of antisense bcl-2 cDNA abolishes tumorigenicity and enhances chemosensitivity of human malignant glioma cells. J Neurosci Res, 2003; 74: 60-6.

46. Mukai S, Kondo Y, Koga S, Komata T, Barna BP, Kondo S. 2-5a antisense telomerase RNA therapy for intracranial malignant gliomas. Cancer Res, 2000; 60: 4461-7.

47. Adachi Y, Chandrasekar N, Kin Y, Lakka SS, Mohanam S, Yanamandra N, Mohan PM, Fuller GN, Fang B, Fueyo J, Dinh DH, Olivero WC, Tamiya T, Ohmoto T, Kyritsis AP, Rao JS. Suppression of glioma invasion and growth by adenovirus-mediated delivery of a bicistronic construct containing antisense uPAR and sense p16 gene sequences. Oncogene, 2002; 21: 87-95.

48. Sehgal A, Ricks S, Warrick J, Boynton AL, Murphy GP. Antisense human neuroglia related cell adhesion molecule hNr-CAM, reduces the tumorigenic properties of human glioblastoma cells. Anticancer Res, 1999; 19: 4947-53.

49. Kondraganti S, Mohanam S, Chintala S K, Kin Y, Jasti SL, Nirmala C, Lakka SS, Adachi Y, Kyritsis AP, Ali-Osman F, Sawaya R, Fuller GN, Rao JS. Selective suppression of matrix metalloproteinase-9 in human glioblastoma cells by antisense gene transfer impairs glioblastoma cell invasion. Cancer Res, 2000; 60: 6851-5.