Brain stem cells and IGF-I: implications in development, regeneration and cancer therapeutics

Silvia J Bueno[1], Annabelle Trojan[2], Ricardo Santander[2], Alvaro Alvarez[3], Andrea Guzman[5], Carolina Rojas[5], Juan J Rey[4] and Jerzy Trojan[5*]

[1]Faculty of Health Sciences, UNAB University, Bucaramanga, Colombia

[2]Faculty of Sciences, UNAB University, Bucaramanga, Colombia

[3]Faculty of Medicine, University of Cartagena, Cartagena de Indias, Colombia

[4]Faculty of Health Sciences, UNAB University, Bucaramanga, Colombia

[5]ICGT and Lab. Legrand S.A., Bogota, Colombia, and NSERM U602, Villejuif, France

Abstract

For the past 40 years, our challenge has been finding a solution for the treatment of brain tumors using our knowledge of the evolution of life, the chemistry of proteins, genetics, and molecular biology. Efficient strategies were established by the construction of vectors targeting oncoproteins or growth factors (i.e. IGF-I, AFP) present during the development of the embryonic and fetal nervous system tissues originating from neural stem cells. The neoplastic stem cells, PCC3 and PCC4, derived from mouse teratocarcinoma – tumor mimicking the structures of developing central nervous system - were transfected with the vectors expressing either antisense IGF-I RNA or IGF-I RNA forming a triple helix RNA-DNA. Both approaches completely stopped the synthesis of the IGF-I growth factor and converted the stem cells into immunogenic cells expressing MHC-I and B7. The immunogenic anti IGF-I transfected cells became antitumor vaccines. The role played by stem cells in the nervous system has motivated us in the search for knowledge focused on self-regeneration and therapeutic strategies. The strategy of Anti IGF-I vaccines was applied with success for therapy of glioblastoma.

Introduction

The brain is plastic and self-regenerative. In neurogenesis: totipotential immature cells – neural stem cells (NSC) exist in the hippocampus and ventricles, they multiply and undergo differentiation and maturation and settle in the brain site where they activate a process of multiple connections (synapses) in their new environment, a process called synaptogenesis. The stem cells of the nervous system, NSC, produce an important impact in the search for new knowledge in brain histology, physiology and pathology, including the neoplastic mechanism and anti-tumor strategy solutions [1-5].

Neurogenesis takes place in the brain when NSC generate new functional neurons and glia considering they are multipotent cells, with the ability to produce different neural lineages [1,6]. This process begins during foetal development and persists in adult life thanks to NSC present in the subventricular zone (SVZ) [7,8] and the sub granular zone (SGZ) of the dentate gyrus of the hippocampus [9]. Neurogenesis is affected by different aspects of diet [10], exercise [11] and sleep [12] due to the association with increased production of Brain-derived neurotrophic factor (BDNF) or in the expression of genes necessary for proliferation and differentiation of NSC.

Development of NSC is related to insulin-like growth factor 1 and its receptor (IGF-1R); considering that is the convergence between an embryonic and tumor development, the IGF-I and its receptor was proposed as a target to treat the progression of the glioblastoma tumor [13,14]. The objective of this review is to describe the NSC in adults, as well as a relationship between IGF-I, IGF-1R and NSC implied during postnatal life development. Moreover, the use of IGF-I and IGF-IR as a target in immunogene therapy of glioblastoma will be also considered.

Mother cells of the nervous system - Neural stem cells

The studies of Ramón y Cajal surprised the world when they demonstrated for the first-time time the complexity of the microscopic neuronal components. Some of their conclusions supported the concept that we are born with a certain number of neurons that remains constant, and then many of these cells die during the course of life, due to an apoptotic process, damage to the endothelium of cerebral vessels or hyperlipidemia. The neurology studies established, for more than 100 years, the dogma that the brain of an adult remains stable, without additive changes in the number of neurons, with a power of processing and memory in irreversible involution, given by a decrease in the neuronal population over time. Humanity adapted to this concept of progressive deterioration, contributing to intellectual unproductivity. In the decade of the 90s, the advances in electron microscopy and molecular biology have changed that paradigm: the existence of totipotent stem cells (NSC) and their multiplication was demonstrated in the hippocampus and ventricles during differentiation process. Moreover, the relation between differentiation process and the growth factors responsible for neuronal maturation, self-regeneration, synaptogenesis, and cerebral plasticity. The latter allows to assume

the functions of neurons affected by injury or disease, permitting the restoration of impaired functions. A review of information on the Neural Stem Cells, NSC, is carried out since 2002 year when the paradigm of the existence and reproduction of neural stem cells is consolidated through new evidence [15,16].

As far as history of nervous system description is considered, Santiago Ramon y Cajal described first the central nervous system (CNS) and neurons in details. In 1965 Altman evidenced that in adult rat's hippocampus exist neurogenesis in the granule cells zone (SGZ) of the dentate gyrus [17] , and also in the ventricular subventricular zone (V-SVZ) of the lateral ventricles [7,18]. The V-SVZ have 4 types of cells: type A cells are neuroblast, type B1 and B2 are astrocytes, type C cells are proliferative precursors, and type E cells are ependymal cells [7,8] admitting that only type B1 have characteristics of NSC. Type B1 cell has end feet on blood vessels. This cell is characterized by glial markers such as glial-fibrillary acidic protein (GFAP), glutamate aspartate transporter (GLAST), and brain lipid-binding protein (BLBP) [8,19] . When B1 cells proliferate, they produce asymmetric division to produce type C cells and renew themselves [20,21]. After production of type C cells, they divide again and produce neuroblast or type A cells. The last ones then proliferate and migrate to the rostral migratory stream (RMS), that leads to the olfactory bulb (OB) [22] where they become GABAergic inhibitory interneurons [7,23] .

As to the role of the dentate gyrus (DG) composed by different layers, one of them is the sub granulate zone (SBZ) where the NSC are concentrated [24]: parvalbumin - expressing interneurons regulate the states of NSC by GABAergic inputs [25], because these interneurons keep constant stimuli of GABA leading to hyperpolarization. When this impulse decrease, NSC transform themselves and become neural progenitor cells, following further transformation into granule cells and integrate the hippocampus circuitry [26]. Different factors influence neurogenesis, that leads NSCs present in the DG and SBZ to astrogliosis, neurogliosis and vascular remodelling [27-28].

The DG aging generates a more active state of NSC, but decreases the proliferation of NSCs and moreover produces in the OB a reduced response to epidermal growth factor signaling [29]. As far as decreasing of NSC is considered, in the experiment of Villeda et al., it was demonstrated that in young mice inoculated with old mice blood it exists a soluble molecule leading to decrease proliferation of NSC, for example CCL11 (also known as exotoxin) [30]. On the other side, there is also an evidence about the increase of TGF and IL-6 generating a pro-inflammatory state [30].

There are different mechanisms explaining neurogenesis and human cognitive functions including an increase in neurotrophic factors (BDNF and IGF-1) [31-33] reducing the time of cell cycle in S-phase [34,35], preventing the death of new neurons or their relation with angiogenesis [36].

Among different factors affecting neurogenesis [37], the immunologic factors play an important role in development of NSC and neurogenesis during adult life, especially cytokines like interleukin-1 (IL-1) [38], tumor necrosis factor alpha (TNF-α) [39], interleukin-6(IL-6) [40], interferon gamma (IFN- γ) and leukemia inhibitory factor (LIF) [41-43]. Related to immune mechanism Insulin-like growth factor-1 (IGF-I) is a polypeptide of 70-amino-acid [44] playing a role in normal NSCs by inducing differentiation, proliferation or survival of neurons in SVZ and DG [45,46,47]. IGF-I activates genes like RIT-1 to produce Ras-related GTPase and increase proliferation of hippocampal neural stem cells (HNSCs) [48-50]. Besides that, it helps a maturation process of transformation NSCs into neurons, which are integrated in dendritic trees [51]. Moreover, it helps in the SVZ, a migration of the NSCs to the olfactory bulb [52,53]. Also the variants of the IGF-1 like MGF induce neurogenesis in DG and in SVZ [54].

Autoregeneration of CNS

In the 2000s, Fred H. Gage described some of the advances in neuroscience in recent years [55-57]. It was discovered that the brain changes throughout life, originating new neurons and their respective synapses with neighboring cells. Self-regeneration and plasticity allow them to repair themselves in the period after an injury or illness, and adapt to a series of circumstances in which an extra capacity is required, there being the possibility of potentiating the brain in its thinking capacity (see references below). The use of drugs that stimulate the brain to replace its own cells (neurogenesis) and reconstruct damaged neuronal circuits are new perspectives that constitute alternatives to the old use of neurotransmitters and cell transplantation in cases of Parkinson's, Huntington's or spinal cord injury, which have resulted poor. Moreover, the discovery has concerned the phenomenon of appearance of new neurons in the brains of adult birds and non-human primates and humans, giving the phenomenon the name of neurogenesis. They found totipotent stem cells that divide periodically, producing other stem cells, new neurons, support cells or glia, and located predictively in the ventricle, hippocampus and olfactory bulb. Whether they are neurons with their respective synapses or glial cells, depends on the site of the brain where they arrive and on their activity, that occurs at the moment of arrival. It takes 1 month, from the moment the new neuron is formed until it becomes a functional cell capable of receiving and sending information. Neurogenesis is a highly controlled complex process; the transformation of totipotent cells in neurons or glia is regulated by the action of the Neurotropic Factor of the Brain and the Factor Similar to Insulin: brain-dependent neurotropic factor (BDNF) keeps neurons alive; the ciliary neurotropic factor (CNTF) protects neurons from death; neurotropin-3 (NT-3) promotes the formation of oligodendrocytes; GDNF), potentiates the motor neurons to form new branches; epidermal growth factor, EGF, an inducer of the division of brain stem cells and fibroblast growth factor, FGF, in low doses keep alive several types of cells; Glial-2 (GGF-2) stimulates beneficial glial production; Insulin-like Growth Factor - 1, stimulates the birth of neurons and glia cells; FGF and EGF participate in the neurogenesis of the hippocampus to reconstruct the necrotic tissue after vascular accidents; FGT and presenilin increase neurogenesis in Alzheimer to reverse the disease. Fred H. Gage has proposed that the following events occur in neurogenesis: Stem cells are the source of new cells. They are periodically divided into 2 main areas: the ventricles which contain the spinal brain fluid (CSF) and the hippocampus, a crucial structure for learning new information and memory. The hippocampus, by producing new neurons, conditions the formation of connections between existing and new neurons, increasing the brain's ability to process and record

new information. The mother cells proliferate producing other daughter mother cells and neuronal precursors, which, in turn, some will become neurons but others in support cells (astrocytes andoligo dendrocytes, glial cells). The newly formed daughter stem cells move away from their progenitors and migrate to the olfactory bulb where 50% will differentiate into neuronal cells. In the adult brain, newborn neurons have been found in the hippocampus and in the olfactory bulb. Researchers are hopeful to be able to induce cerebral self-repair, stimulating the multiplication of NSCs and the synthesis of neural precursors and their subsequent maturation and specialization in

mature neurons that establish several synapses with other neurons in a determined site, where necessary [6,25,32,36,46,55-60].

Stem cells, neural and glial cells in neoplasia: Therapeutic implications

The stem cells of adult CNS can produce new neurons and cells of the glia: the embryogenic stem cells, isolated in human embryos of only 100 cells, can generate any type of cells of the body [6,25,55,56]. These stem cells can be cultured, multiplied and then transplanted into the Central Nervous System of an adult animal. They survive successfully in the hippocampus and olfactory bulbs and differentiate into mature neurons. If they are placed in another place they do not differentiate in neurons but in glial cells.

In 1979, the first description of the development of the central nervous system using a new marker, alpha-fetoprotein, was made using a rat brain fetal model [61]. Using the same marker, a neoplastic model of CNS development in mouse teratocarcinoma, derived from stem cells PCC3 and PCC4, was described [62]. The comparison of the results obtained in two models, showed that there is a convergence between embryonic fetal development and neoplastic development. Is AFP a perfect antigen for targeting malignant CNS tumor?. Taking into account that AFP is present not only in glial but also in neuronal cells of differentiation, which limits its useful for differentiational diagnosis and therapy of glial and neuronal derivative tumors. Fortunately, a new oncoprotein, the growth factor IGF-I, presented only in glial cells of normal or neoplastic development, but absent in neuronal cells, was considered for therapeutic purpose [63,64]. IGF-I is considered as the most important growth factor of normal and neoplastic development, including CNS [65]. Logically, to stop the neoplastic development, the arrest of the synthesis of IGF-I in cancer cells of glioblastoma, in the "source" at the level of transcription or translation was considered, using antisense [66–68] or triple helix [69,70] technology.

The translation level of IGF-I was targeted in glioma malignant cells by antisense approach using a vector expressing antisense IGF-I RNA [64]. This technology has yielded positive results *in vitro*, stopping the synthesis of IGF-I in glioma cell cultures, and *in vivo*, stopping the neoplastic development of the tumor [71]. This strategy has given historically the birth of a new oncology domain - cancer gene therapy [72]. The injection *in vivo* of transfected anti – gene (antisense or triple helix) anti IGF-I cells either into animals carrying teratocarcinoma tumors derived from stem cells PCC3 and 4, or into glioma rat tumors using also transfected cells, or applied in the clinical treatment of patients with glioblastoma, has induced an antitumor immune response mediated by TCD8 + cells [71,73-75].

We illustrate below the efficiency of molecular biology applying anti – gene strategy targeting the IGF-I in the model of mouse teratocarcinoma derived of stem PCC3 and PCC4 embryonal carcinoma stem cells (the technique of transfection of PCC stem cells using either antisens or triple helix vectors [73,74] (Figures 1 – 8). We need to add, that an equal to the genetically modified PCC stem cells, does not exist in nature.

The proposed mechanism of anti – gene (antisense or triple helix) therapy concerns the growths factors and their receptors (IGF-I, TGF-beta, EGF, IGF-I-R, EGF-R) – a combination of an increased anti-tumor immune response (CD8 +), and an inhibition of the transduction pathway of the PI3K / AKT / GWK3 / GS signal that is involved in the transformed phenotype of the tumor [76]. In glioblastoma, glial tumor cells proliferate, forming rapidly growing tumors. How these cells
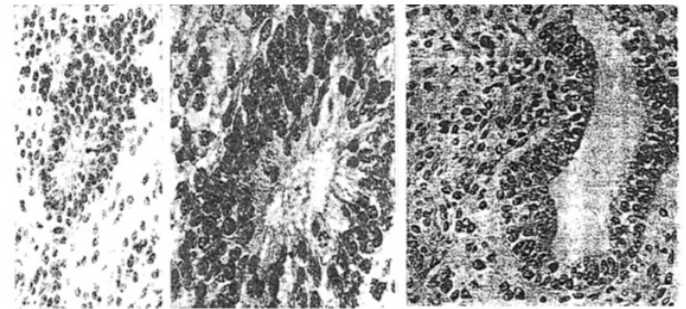

Figure 1. Teratocarcinoma structures resulted of injection into 129 mice of stem cells – PCC3 embryonal carcinoma cells. (left) Neuroepithelial rosette surrounded by neuroepithelial cells, HE, x250; (middle) Higher magnification of the same poorly differentiated neuroepithelial rosette, x400. (right) More advanced step of neuroepithelial differentiation showing a cyst of nervous origin ''pathological neural tube''. The wall of the cyst as well its neighborhood is constituted by the same type of neuroepithelial cells. Neuroepithelila cells surrounding the cyst present a character of neurospongium, HE, x250 .
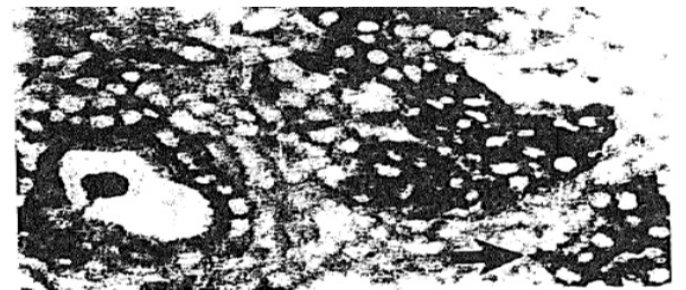

Figure 2. Teratocarcinoma resulted of injection of stem cells, PCC3 cells, showing an intermediate step of cyst differentiation (bottom down) if compared to two structures presented in figure 1. The differentiating neuroependymal tube and groups of cellas arranged in clusters (arrow) are labelled with antibodies anti AFP, Immunoperoxidase, x250.

Figure 3. Teratocarcinoma resulted of injection of stem cells - PCC3 embryonal carcinoma cells. Sequence of maturation of neuroepithelial structures showing steps of differentiation: 1. In differentiated; 2. Poorly differentiated (rosette); 3. Moderately differentiated; 4. Well differentiated, immature - embryonic form); 5. Well differentiated, immature – fetal form; 6. Well differentiated, mature. End points of AFP staining shows maximum in the steps 3 and 4; AFP is absent in steps 1 and 6.

originate from the mother stem cells cells induced by the elements that regulate their division, would be promising for this pathology.

IGF-I is considered to play a principal roll in tumorigenesis [65] and especially in glioblastoma - the most common primary brain tumor in adults [77] , with a prognosis for survivals below 1 year, and only in 5-10% cases below 2 years [78]. In this tumor, as well as in teratocarcinoma derived of PCC stem cells, the IGF-I and insulin increases proliferation by different signalling pathways, promoting cell growth of glioblastoma [79]. By the way, the clinical epidemiologic studies evidenced an increase mortality rate in patients treated with insulin [80], underlying the supplement data of signal transdution pathway of IGF-I playing a role in risk factor of the cancer development [79, 81]. A serum level of IGF-I is higher in glioblastoma patients [82], and tumor tissues present an increased IGF-I and insulin receptors levels compared to normal tissue [83] inducing the proliferation, differentiation and migration of tumor cells [83,84]. The mechanism of mutation in p53 inducing up-regulation in IFG-I receptor, and for the same reason a survival of cancer cells, can play also a role [85].
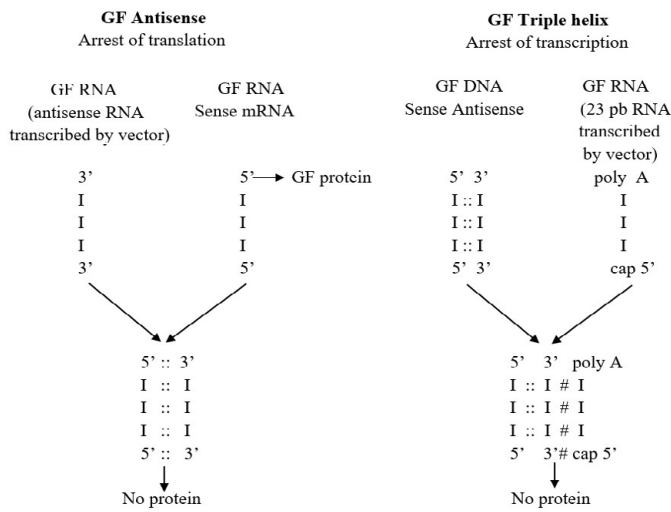
Figure 4. Schema of Antisense and Triple helix technology to arrest Growth Factor, GF. In antisense technology the end result is the inhibition of GF mRNA (sense RNA) activity by binding to the antisense RNA. In GF triple helix technology, the oligopurine third strand (23bp) forms RNA-DNA triple helix with GF gene.
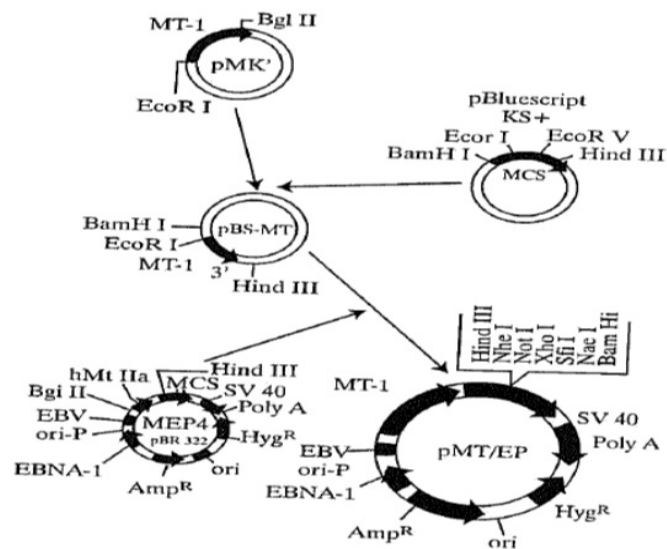

Figure 5. Schema of preparation of pMT/EP vector for IGF-I antisense and IGF-I triple helix technology.

New or proposed therapies of glioblastoma are based either on immune treatment or on immuno-gene strategies [86], including inhibitors [87], and the anti – gene strategy [66-70,88]. The last approach has permitted us to establish new and successful gene therapy strategies targeting glioma's growth factors and have now been introduced into clinical trials (the median survival of patients has reached 2 years, and in some cases 3 or 4 years) [89].

Other AS approaches targeting TGF-beta or VEGF, their receptors and their down stream transduction signaling elements [90-92], appear to offer hope for a promising solution. The recent neuro-oncology research underlines a focus on the role of the PI3K/AKT pathway in glial cells: the simultaneous arrest of at least two links either IGF-I or TGFbeta or VEGF and GS, of the pathway TK/PI3K/AKT/GSK3/GS [92-94] seems to be in line for a future clinical gene therapy trial strategy for treatment of GBM.

Other recently introduced technologies include potentially useful siRNA [95,96] and miRNA (microRNA) [97]. The role of 21-23 mer double-stranded RNA (si RNA) in the silencing of genes is strongly similar to that of the TH DNA mechanism, which also involves 23 mer RNA [70]. As to miRNAs, they may play a fundamental role in tumorigenesis, controlling cell proliferation and apoptosis; in gliomas, the miRNA (microRNA-21) level has been reported to be elevated [98].

New horizons: Stem cells of SNc and learning

Synaptogenesis plays an important role in learning and memory. It takes a month to move from newly formed daughter stem cells to the integration of daughter cells in a brain circuit and connect with existing ones, forming synapses. In the process of synaptogenesis, the dendrites of one neuron are contacted with the axon of another neuron. Dendritic spines change their shape in minutes. In his article Fred Gage informs us: "Exercises, physical activity, experience, can regulate the rat of neurogenesis in the hippocampus, the subsistence of newborn stem cells neurons and their ability to integrate into the existing neuronal circuit "[56]. Henrieth van Praag has showed that a mouse exercised on a rotating ring can double the number of dividing stem cells in the hippocampus [99,100].

Concerning the mechanism of memory, scientists investigate small molecules that can stimulate genes that produce growth factors. Another possibility is to use a gene therapy and cell transplantation. These technics could permit to increase a cellular production of growth factors by genetic manipulation followed by the implant of these cells

3' IGF-I 5' cDNA in antisense orientation
(ANTISENSE TECHNOLOGY)

or

5' CTGGAAGCTT TCTT CTCCCTCTCTCTCTCTTCCGGATCCCCTC
3' GACCTTCGAA AGAA GAGGGAGAGAGAGAGAAGGCCTAGGGGAG
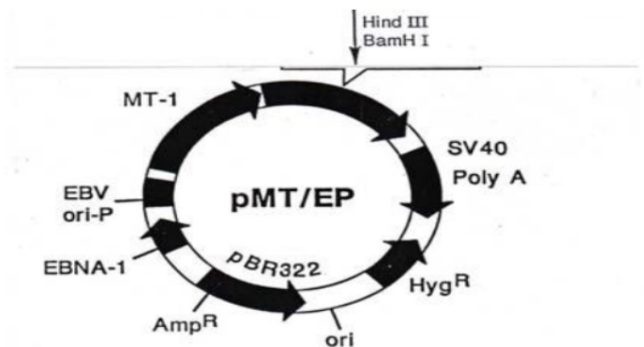
(TRIPLE HELIX TECHNOLOGY)

Figure 6. Construction of antisense and triple helix vectors. In antisense technology cDNA in antisense orientation is inserted: the episomal vector express antisens IGF-I RNA. In triple helix technology the 23 bp RNA prepared by PCR is inserted: the episomal vector express 23 bp RNA forming triple helix with genomic DNA as follows:

AGAAGAGGGAGAGAGAGAAGG -- oligopurin poli A 3' AGAA 5' cap
****
AGAAGAGGGAGAGAGAGAAGG
IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII -- genomic DNA
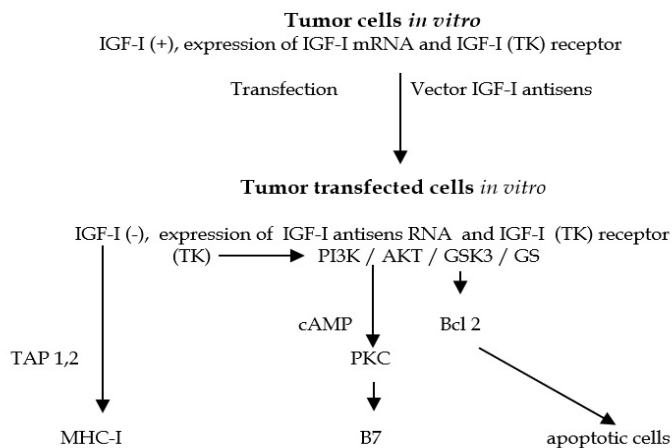TCTTCTCCCTCTCTCTCTCTCC
***Hoogsteen bonds
III Watson-Crick bonds

Figure 7. Mechanism of immunogene therapy. After transfection *in vitro* of stem cells - PCC3 embryonal carcinoma cells, using Antisens IGF-I vector, the cells stop to synthesize IGF-I, and become immunogenic, expressing MHC-I and B7 antigens. One part of cells enter in apoptosis Celulas "antisentido" (anti IGF-I). The injection of these transfected cells into animal induce anti tumor immune response mediated by CD8 lymphocytes. [87,93]. Abreviaturas: TAP 1,2: transporter associated withantigen processing antigen); TK: tyrosine kinase; PI3K: phosphatidyinositol 3 kinase; PDK1: phosphoinositide-dependent kinase 1; AKT: PKB, protein kinase B; Bcl 2: key molecule of apoptose; GSK3: glycogene synthetase kinase 3; GS: glycogene synthetase; PKC: protein kinase C.
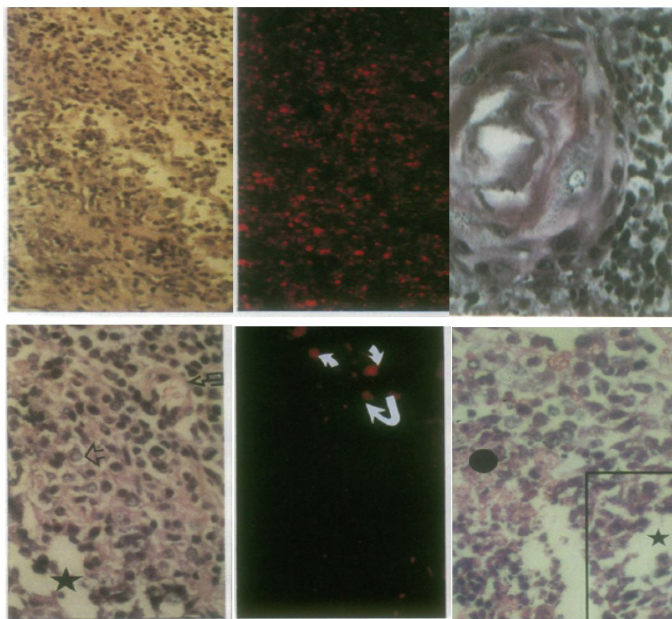

Figure 8. (Up) Lesions resulting from injection of PCC3 cells expressing antisense IGF-I RNA (left and middle) and antisense IGF-II (right) into 129 SV mice. (left) Lymphocyte infiltration, HE, x80; (middle) serial section stained with anti CD8 antibodies, x80. (right) Mature epidermoid structure accompanied by a sheet of dark neuroepithelial cells, HE, x125. (Down) Tumor regression induced by injection of antisense IGF-I expressing PCC3 transfected cells. (left) Teratocarcinoma 4 days following transfected PCC3 cells in hection, showing embryonal carcinoma cells (open arrow), neuroblastic cells arranged in pseudo rosettes (star), and nervous-system derived cells scattered about blood vessel (broken arrow), HE, x110. (middle) Serial section stained with anti CD8 antibodies, x110. (right) Teratocarcinoma 9 days following transfected cell injection, showing embryonal carcinoma cells and pseudo rosettes of neuroblastic cells (star), and disentegrating and necrotic tissue (black circle), HE, x110.

in a certain region of the brain. This possibility requires an ethical consideration: the growth factors could induce tumor development, and the transplanted cells could be out of control. That would be an acceptable risk in patients with Alzheimer's disease, but not in healthy ones.

Environment stimulates neurogenesis in the ventricles and the hippocampus, the migration of stem cells and neuronal precursors to the olfactory bulb. Then the stem cells 'travel' to specific sites within the brain where synaptogenesis will occur, participating in events that generate plasticity and capacity for self-regeneration and brain self-repair. Ratey tells us that "aging is inevitable, a serious loss of memory is not" [101]. Several factors can delay the onset of memory impairment in Alzheimer's and senile dementia; there are, among others, following subjects in current research:the ingestion of antioxidants such as vitamin E and Celegeline, nonsteroidal anti-inflammatory drugs, Aspirin and Ibuprofen, vitamin B 12, ingestion of Carnitine. Ginkgo biloba has not been shown to have favorable effects on neurogenesis, but it can increase blood flow and cerebral oxygenation [16,56,99-106].

Authorship and contributorship

Acknowledgements

Funding information

Competing interests

The authors declare that they have no competing financial or non-financial interests.

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