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Perspectives of Stem Cell-Derived Microglia for Medicine

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1. Introduction

Microglia, the resident immune cells of the central nervous system (CNS), are responsible for the innate brain immune defence. The exact origin of microglia is still unclear, but several reports suggest that microglia are of myeloid origin (Chan et al., 2007). They appear for the first time at an early embryonic state in the neuroepithelium and populate the brain from the blood in a second perinatal phase. Under pathological conditions or infections, microglia migrate to the affected tissue. On site, they change their phenotype to a pro-inflammatory cell type, release cytotoxic molecules such as reactive oxygen species to fight against microbes or clear tissue debris after sterile injury. Microglia also phagocyte oncogenically transformed brain cells and are able to activate additional immune cells. Pro-inflammatory microglial cells are also involved in the progression of neurodegenerative diseases.

Research on microglia is mainly performed on primary cells. However, only a limited amount of murine or human microglial cells can be obtained from the brain tissue, thus complicating investigations of drug screenings or new cell therapy approaches that are requiring a high number of cells. For this reason, stem cell-derived microglia represent a useful tool for further studies to elucidate the role of microglia in diseases and therapeutical approaches. Data from others and our laboratory show that microglial precursor cell lines can be obtained from mouse embryonic stem cells or human induced pluripotent stem cells via a neural differentiation protocol. Human microglial cell lines open new alternatives for drug screening, combating cancer and regenerative approaches for neurological diseases.

2. Microglia in neuroinflammation and neurodegeneration

The CNS contains two major cell types: neurons and glial cells. Neurons build a network throughout the nervous system and forward information through electrical conduction. Glial cells are only indirectly involved in the conduction process, but play an essential supportive role to neurons. Glial cells of the CNS can be subdivided into two main classes: macroglia consisting of astrocytes and oligodendrocytes and microglia. Microglia are the only immune competent cells in the intact CNS. They were defined by Ramon Y Cajal in 1913 as a third group of cells in the CNS and termed mesoglia (Rezaie and Male, 2002). Microglial cells were further characterized by Rio del Hortega using silver carbonate staining (del Rio-Hortega, 1933). He defined the term microglia and proposed that they can

change their morphology dependent on their activation state. Microglial distribution varies by brain region, but they predominate in the grey matter with highest concentrations in the hippocampus, the substantia nigra, the olfactory telencephalon and the basal ganglia (Block et al., 2007). In the healthy CNS the so called 'resting' microglia display a ramified morphology. However, microglial cells are not resting at all, but continuously survey their microenvironment with their processes. Time-laps imaging using *in vivo* two-photon microscopy revealed that the somata of microglial cells remain stably localized whereas the processes are remarkable motile (Nimmerjahn et al., 2005). The microglial processes show continuous cycles of withdrawal and new formation, thereby scanning the whole brain parenchyma every few hours. Upon immunological stimuli or in response to brain injuries microglia become activated and migrate towards the stimuli. At the lesion site they change their phenotype from a ramified to an amoeboid morphology, clear debris and apoptotic cells and promote tissue repair. It was reported that microglia increase neuronal survival through the release of trophic and anti-inflammatory factors (Block et al., 2007; Streit, 2002). In their activated state microglia up-regulate several surface molecules such as CD14, major histocompatibility complex (MHC) molecules, complement receptors and chemokine receptors (Rock et al., 2004). Furthermore, pro-inflammatory activated microglia release a wide range of soluble factors like superoxide, nitric oxide and tumor necrosis factor-α (TNFα), which have neurotoxic effects on neurons. It seems that microglia have both, neuroprotective and neurodestructive functions. In contrast to acute CNS injuries, chronic diseases are characterized by slow progressive neurodegeneration that takes years to develop (Streit, 2002). There is strong evidence that microglial cells are involved in a wide range of neurodegenerative diseases (Block and Hong, 2005) like Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD). Actually, AD was one of the first neurodegenerative diseases associated with neurotoxic microglia (Block and Hong, 2005). AD is a neurodegenerative disorder leading to cognitive, memory and behavioral impairments. The pathological hallmark of AD consists of cortical atrophy with accumulation of extracellular deposits of amyloid β (Aβ) in senile plaques and intracellular neurofibrillary tangles (NFT) of hyperphosphorylated tau protein in the cerebral cortex (De Strooper and Annaert, 2000). Senile Aβ plaques recruit and activate microglia, which in turn produce pro-inflammatory factors that act neurotoxic on neurons. Damaged neurons activate microglia independent of the mode of damage. Finally, this leads to neurotoxin producing microglia resulting in a perpetuating toxicity.

3. Origin and sources of microglia

During embryonic development gastrulation conduces to the creation of the three germ layers: ectoderm, mesoderm and endoderm (Arnold and Robertson, 2009). The ectoderm gives raise to the peripheral and central nervous system as well as to the epidermis structures. The mesoderm which is located between the endoderm and ectoderm develops into blood, muscles, bone, cartilage, notochord and the connective tissue. Organs associated with the digestive system such as pancreas, liver and the epithelium of the digestive and respiratory system are of endodermal origin.

The origin of microglia is still under debate due to the lack of a specific microglial marker. There are different hypotheses about the origin of microglia. In previous times some scientists held the view that microglia are of neuroectodermal origin and are derived from either the ventricular zone of the lateral ventricle or glioblasts (Kitamura et al., 1984;

Paterson et al., 1973). Kitamura and colleagues (Kitamura et al., 1984) used 3H-thymidine autoradiographic analysis to investigate the genesis of microglia within the mouse hippocampus. They concluded that resting microglia originate from glioblasts, direct derivatives of matrix cells, that are of neuroectodermal origin. Interestingly, the possibility that microglia might be derived from the neuroectoderm via a mesenchymal stem cell type was supported by a recent publication (Takashima et al., 2007). Embryonic stem cells were cultivated and differentiated under two different conditions, one gave rise to mesodermal cells and the other one generated cells from the neuroectodermal lineage. Takashima and colleagues could show that mesenchymal stem cells (MSC) do not develop from mesodermal cells but from cells positive for Sox1, a molecular marker specifically expressed on neuroepithelial cells. They concluded that this way is representing the earliest recruitment of MSCs, whereas the origin of the later waves remains unsolved.

However, in 2002 it was shown that microglia express the transcription factor PU.1, which is myeloid specific. Therefore, it was suggested that prenatal microglia are derived from mesenchymal cells from the myeloid lineage (Rezaie and Male, 2002). Various monoclonal antibodies that are used to identify monocytes and macrophages like CD11b, CD45, CD68 and MHC class II also label microglia which supports the notion that microglia are derived from blood monocytes or another monocyte-related myeloid cell type. This view was further supported by the discovery that the first appearance of microglial cells coincide with the vascularization of the brain. It was suggested that circulating monocytes enter the nervous system and transform to microglia (Imamoto and Leblond, 1978; Ling, 1979) before the formation of the blood-brain-barrier has been completed (Perry et al., 1985). Infiltration of monocytes could be confirmed by carbon-labelling (Ling et al., 1980) and histochemical studies using marker enzymes for monocytes (Ferrer and Sarmiento, 1980; Ling et al., 1982).

Andjelkovic and colleagues labelled microglia in the developing and adult human brain using lectin-histochemistry (Andjelkovic et al., 1998). Lectin positive cells can be subdivided into two groups which both exist in the adult brain, but show different distribution and time schedule of morphological changes. Their results support the consideration that there could be different origins of microglia.

Two questions remain to be answered: when do the first microglia appear and what is their origin. It has been established that microglial progenitors populate the nervous system during embryonic and fetal development (Rezaie and Male, 1999). Some authors have suggested the yolk sac as the tissue of origin for all tissue macrophages during embryonic development (Alliot et al., 1999; Kaur et al., 2001). Alliot and colleagues (1999) reported that cells with properties of microglial precursors can be first detected at embryonic day E8 in mice and that the majority of these progenitors originate from the yolk sac.

Nowadays, it is suggested that microglia populate the brain in two waves. The first one takes place in the neuroepithelium during embryonic development around E8-9 in rodents with unknown origin (Alliot et al., 1999). The second wave occurs in the brain during fetal development at E17-18. These cells are derived from a pool of circulating myeloid precursor cells of mesodermal origin (Chan et al., 2007).

In the adulthood, it is widely accepted that microglia invading the diseased CNS are derived from circulating monocytes originating in the bone marrow (Perry et al., 1985; Rezaie and Male, 2002). It was described that in bone marrow chimeric mice circulating monocytes are recruited to the lesion site of the brain and differentiate into microglia (Mildner et al., 2007).

However, it seems that the inappropriate stimulus of irradiation is required for the recruitment (Mildner et al., 2007).

Microglial function is often studied on primary microglial cells, which are isolated and enriched from mixed glial cultures derived from the brains of postnatal mice or rats. But the cell number obtained by a shaking procedure from mixed glial culture flasks is rather low (Giulian and Baker, 1986). Optionally, a pure population of microglial cells can be obtained using a combination of density gradients and flow cytometric sorting (Ford et al., 1995). This methodology is applied to isolate both mouse and human microglia. However, the obtained number of primary microglia is very limited in rodents and humans. This aspect complicates classical biochemistry studies, systematic screening tests or cell therapy approaches. Therefore, the BV2 cell line which is originated from primary mouse microglia by oncogenetic transformation (Blasi et al., 1990; Bocchini et al., 1992) is used as a substitute for primary microglia. Murine cultured microglia are immortalized via injection with v-raf/v-myc recombinant retroviruses. The obtained BV2 cells show properties like macrophages in terms of antigen profile and phagocytic capacity. However, due to the transformation process these cells show an altered cytokine profile and changes in their migratory capacity (Horvath et al., 2008; Napoli et al., 2009). Later on, Nagai and colleagues established an immortalized cell line of human microglia termed HMO6 (Nagai et al., 2005; Nagai et al., 2001). This cell line is developed from human embryonic telencephalon tissue by retroviral transformation with v-myc. Certainly, these cells can not be used in therapeutical approaches due to the usage of retroviruses for transformation. Therefore, microglia derived from embryonic stem (ES) cells might provide a new tool to study microglial function and to apply them for cellular therapies.

4. Embryonic and induced pluripotent stem cell-derived microglia

During the last decades a major breakthrough was the development of mouse pluripotent embryonic stem cell lines (Evans and Kaufman, 1981). These cells are isolated from the inner cell mass of embryos and can be cultivated *in vitro* on a feeder layer of mouse embryonic fibroblasts (MEF) in the presence of the leukemia inhibitory factor (LIF). In addition to the MEF, which are supposed to secrete crucial factors to promote self-renewal, cultivated murine ES (mES) cell lines also require the presence of LIF to maintain their undifferentiated stage (Smith et al., 1988; Williams et al., 1988). In culture, mES cell lines show an almost unlimited proliferation capacity and maintain nearly a normal and stable karyotype (Smith, 2001). During cultivation ES cell lines do not loose their pluripotency and are able to generate all cell types of an embryo (Wobus and Boheler, 2005).

Due to their crucial role in neurodegenerative diseases microglia could be used as a cellular vehicle for gene or regenerative therapies in the CNS (Neumann, 2006). The CNS is delimited from the circulating blood by the blood-brain-barrier which controls the entry of substances and molecules. Theoretically, only 2 % of small molecules are able to enter the CNS, whereas large molecules are unable to pass the blood-brain-barrier. Therefore, only a limited number of drugs can be introduced in CNS diseases. Microglial cells are a migratory cell type that is able to pass the blood-brain-barrier and could therefore be used as a vehicle to introduce therapeutical proteins into the CNS to the affected tissue.

As already discussed, the obtained number of primary microglia is very limited in rodents and humans using the before mentioned protocols. Contrary to these methods, the generation of microglia derived from pluripotent stem cells constitute a source of unlimited

numbers of microglia which can be used for biochemical studies, systemic screening tests and cell therapy approaches. Recently, a protocol for the generation of microglia derived from mES cells was developed (Beutner et al., 2010; Napoli et al., 2009). This effort was initiated by a work from Tsuchiya and colleagues who succeeded in the differentiation of microglial-like cells derived from mES cells (Tsuchiya et al., 2005). During their neuronal differentiation based on a protocol for the generation of dopaminergic neurons (Lee et al., 2000) they found a population positive for Iba1 and CD45. Using a density gradient, these cells with morphological characteristics of primary microglia could be isolated. However, these isolated cells were not described to survive and proliferate. Thus, we generated a protocol to obtain microglial precursors from mES cells (Beutner et al., 2010; Napoli et al., 2009). These ES cell derived microglial precursors (ESdM) are stable proliferating in culture and have substantially most characteristics of primary microglia. The differentiation of mES cells to microglial precursors requires a five step protocol (Beutner et al., 2010). ES cells are cultivated on an irradiated MEF layer in defined medium in the presence of LIF. To induce spontaneous differentiation, ES cell clusters are detached and cultured on non-adherent culture dishes without LIF. So called embryoid bodies (EBs) are generated and plated on gelatine-coated cell culture dishes in ITS-medium supplemented with fibronectin. Under these conditions cells start to proliferate and different cell types develop. Most of these cells can be immunostained against the intermediate filament protein nestin which is mostly expressed by neuronal precursors. After 6 days in this selection stage medium is changed to N2-medium to expand cells in the presence of FGF2 and laminin for 6 more days. Withdrawal of FGF2 induces differentiation of neurons, astrocytes and oligodendrocytes (Beutner et al., 2010). Within this mixed cell culture cells with microglial morphology can be detected around three weeks after removal of FGF2. The proliferation of these cells can be enhanced through addition of granulocyte macrophage colony stimulating factor (GM-CSF). Microglial precursors are manually isolated and cultivated in N2-medium. They attach to the cell culture dish and typically follow an exponential growth rate. Expression of the marker Iba1 and CD68 indicate that the isolated cells are microglial precursor cells derived from mouse embryonic stem cells. Comparable to primary microglia ESdM show high expression of CD11b and CD45 (Beutner et al., 2010). Analysis of α4 integrin and β1 integrin show high expression levels of these two integrins by ESdM and primary microglia. In contrast, the expression of CD34 and cKit which are both expressed on certain stem cell populations can not be confirmed indicating that ESdM cell lines lost their stem cell properties. Besides, ESdM show expression of B7.2, a costimulatory ligand that is expressed on antigen-presenting cells and that together with its receptor is responsible for T and B cell activation. In addition, a weak expression of major histocompatibility complex (MHC) class II which is also expressed on antigen-presenting cells can be confirmed. Up to passage 20 no relevant changes of the surface marker profile can be observed. However, from passage 25 onwards ESdM lines show a decreased expression of the analyzed markers indicating that the phenotype is not stable over very long proliferation periods.

A typical hallmark of microglia is the inducibility of pro-inflammatory cytokines and reactive oxygen species after stimulation. ESdM cell lines and primary microglia stimulated with lipopolysaccharide (LPS) show an upregulation of inducible nitric oxide synthase (iNOS) and interleukin 1β (IL-1β) transcript levels (Beutner et al., 2010; Napoli et al., 2009). Interferon-γ (IFNγ) stimulation results in an increase in iNOS transcripts in ESdM comparable to primary microglia whereas IL-1β gene transcription is almost not affected in both cell types. Furthermore, a chemokine assay determines that ESdM migrate towards the chemokine

CX3CL1 in a dose-dependent manner (Beutner et al., 2010). Comparable to primary microglia, migration is significantly increased following a CX3CL1 gradient. The phagocytic capacity is an important aspect of microglia. After LPS stimulation ESdM show an increase in phagocytosis of microsphere beads in comparison to unstimulated cells (Beutner et al., 2010).

In summary, ESdM show most characteristics of primary microglia and therefore can be seen as a new promising tool to study microglial function *in vitro* and *in vivo*.

In 2006, Takahashi and Yamanaka identified 24 potential candidate genes that were thought to be involved in maintaining pluripotency and self renewal of ES cells (Takahashi and Yamanaka, 2006). They showed that reprogramming of mouse embryonic fibroblasts to an embryonic-like state is possible after retroviral transduction with the four factors Oct4, Sox2, Klf4 and c-Myc. These so called induced pluripotent stem (iPS) cells showed similarities to ES cells in terms of morphology, proliferation and expression of pluripotency markers. Furthermore, iPS cells are able to differentiate into all three germ layers and therefore can be used to generate every cell type of the body. Some month later different groups succeeded in the generation of iPS cells from human adult fibroblasts (Takahashi et al., 2007b; Yu et al., 2007). Fibroblasts are obtained from human skin tissue biopsies and cultured under defined conditions. For reprogramming, human fibroblasts are retrovirally co-transduced with Oct4, Sox2, c-Myc and Klf4 genes. The obtained iPS cells are cultured according to protocols established for human embryonic stem cells. The analysis of iPS cells shows that they display a normal morphology, a normal karyotype and the expression of several pluripotency markers like Oct4, SSEA-3, SSEA-4, Tra-1-60 and Tra-1-81. Furthermore, multilineage differentiation of iPS cells is confirmed by embryoid body and teratoma formation. However, the generation of iPS cells using retroviruses includes the integration of genetic material and thereby the potential risk of genetic modifications of the target cells. To overcome this issue modified methods were developed to generate iPS cells (Yamanaka, 2009). Recently, it was shown that proteins conjugated with a short peptide mediating protein transduction can be directly delivered into the target cell. Using recombinant cell-penetrating reprogramming proteins, iPS cells are obtained from mouse fibroblasts (Zhou et al., 2009).

In a next step, human iPS cells are used for differentiation into microglial precursors (unpublished, pending European patent EP2010_055731). A differentiation protocol is applied with nearly the same five steps described for the generation of ESdM. Human iPS cells are cultured on a layer of irradiated MEF in the presence of FGF2. After some days in culture, iPS colonies can be detached and cultivated on non-adherent dishes without any growth factors to induce spontaneous differentiation. The resulting EBs are allowed to attach to the culture dish and grow out in defined medium supplemented with FGF2 and fibronectin. For expansion of the cells medium is changed to N2-medium in the presence of FGF2 and laminin. Withdrawal of the growth factor after some days induces the differentiation of microglial precursors identified by morphology. Microglial identity is confirmed using immunocytochemical analysis with antibodies against the microglial marker Iba1 and CD68. The iPS-derived microglial precursor (iPSdM) cells can be isolated and cultivated. Because disease-specific iPS cells (Dimos et al., 2008; Ebert et al., 2009; Soldner et al., 2009) can be generated, this method might represent a new tool for regenerative medicine for study and treatment of disease models.

5. Target pathway identification and drug screening

Drug discovery processes are costly in terms of time and expenses. High throughput rate screening technologies allow rapid testing of compounds, but might lack physiological

conditions. Classically two distinct sources of human cell types, primary or immortalized cell lines, are available for drug screening technologies (Ebert and Svendsen, 2010). Primary cell lines are fully differentiated cell types with close approximation of native function, but often are inaccessible, require fresh preparation and have questionable reproducibility. In principal, primary microglial cell lines are not available due to the inaccessibility of the human brain tissue and difficulty to maintain the cells in culture.

In contrast, immortalized cell lines have low costs for maintenance and are homogenous cell populations, but lack important aspects of native cells such as certain metabolic or migratory functions. Furthermore, many cell types are not available as immortalized cell lines. In literature, a human immortalized microglia cell line was described (Nagai et al., 2005; Nagai et al., 2001). This cell line (HMO6) is developed from human embryonic telencephalon tissue by retroviral transformation with v-myc and is reported to possess most immunological and functional characteristics of primary microglia. However, in comparison to primary microglia, HMO6 cells display an altered cytokine profile (Nagai et al., 2001). Therefore, they probably do not fully reflect the properties of primary microglia.

Now, a new technology to obtain human microglial cells becomes available for drug screening technologies. As described above for microglia, embryonic stem cells or induced pluripotent stem cells can be differentiated into stably proliferating cell types. Most adult tissue cell types including microglia can be obtained at high quantities using this technology. The tissue cell types differentiated from stem cells show functional properties very close to the native cell types. Most interestingly, cell lines can be generated from diseased patients, too. However, reproducible methods to obtain fully differentiated cells and to achieve purified populations are still under development. As described above, microglial lines now can be generated from human induced pluripotent stem cells, which reflect very much the native function of primary microglia. The microglial lines can then be used for screening technologies.

One possible use of human microglial cell lines could be screening tests to evaluate the effect of new possible drugs or substances that might act toxic. Nanoparticles which are sized between 1 and 100 nm are basically of interest in pharmacy and medicine as the number of products containing synthesized nanoparticles steadily increases. Orally absorbed nanoparticles via drugs or food can pass the blood system via the gut. It was reported that silica crystals can activate immune cells, are phagocytosed and transported into the lysosome (Hornung et al., 2008). Given that these nanoparticles can not be digested, lysosomal swelling and damage might be induced in phagocytes including microglia. In addition, lysosomal damage activates the inflammasome and the release of the pro-inflammatory cytokine IL-1β. It is expected that the size, structure and type of nanoparticles might influence this lysosomal toxicity. Interestingly, aggregated amyloid β (Aβ) which is involved in the pathology of AD and exhibit a size of 10 – 200 nm can activate microglia in a similar way (Halle et al., 2008). Halle and colleagues demonstrated that microglia phagocytose fibrillar Aβ and transport it into the lysosome, where it induces swelling and damage of this organelle. The inflammasome of microglia is activated which leads to the release of inflammatory mediators like IL-1β. Furthermore, diesel exhaust particles (DEP) mediate the neurotoxicity on dopaminergic neurons caused by microglia, possibly by a similar mechanism (Block et al., 2004). Diesel exhaust particles uptake leads to the activation of microglia and their production of reactive oxygen species and nitric oxide, which induces toxicity of dopaminergic neurons. In summary, nanoparticles and other small particles principally have the risk to activate microglia leading to the release of pro-inflammatory

cytokines and radicals which can promote the pathology of neurodegenerative diseases. Thus, microglial lines could help to evaluate the possible brain toxicity of very small particles.

Another possibility to use human microglial cell lines for screening is the development of novel drugs for neurodegenerative diseases. Activated microglia are reported to be involved in the pathology of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). Approximately, 10 – 20 % of patients with familiar ALS show a mutation in the superoxide dismutase-1 (SOD1) gene whose product is involved in the conversion of free superoxide radicals. In an animal model of ALS it was demonstrated that microglia with mutant SOD1 drive the disease onset and progression in particular via the release of pro-inflammatory molecules and radicals (Boillee and Cleveland, 2008). Mutant SOD1 stimulates the NADPH oxidase resulting in an overproduction of reactive oxygen species (ROS). Therefore, it is proposed that ROS produced by microglia mediate the pathogenesis of familiar ALS associated with a mutation of SOD1.

Human iPS-derived microglia cell lines would provide a new possibility to investigate the effect of different SOD1 mutations on the toxicity of microglia. Therefore, iPS-derived microglia could be transduced with mutant SOD1 or obtained from patients with a SOD1 mutation and analyzed for abnormal radical and pro-inflammatory cytokine production. Furthermore, co-culture of those genetically modified or mutated microglia and motor neurons could give more insight in the pathology of the disease.

Since iPS cells can be generated from diseased patients (Dimos et al., 2008; Ebert et al., 2009; Park et al., 2008) they represent a new tool for disease modelling screens of several neurodegenerative diseases. Ideally, iPS cells are expanded and differentiated in the disease relevant cell type such as microglia. The differentiated cell type would allow drug screening tests and investigations of genes differently regulated or mutated in this cell type.

In summary, iPS-derived microglia represent human conditions better than other cell lines, thus allowing drug screening and identification of pathways affected in neurodegenerative diseases.

6. Combating cancer

The most common and aggressive type of primary brain tumors in humans is the glioma, which most probably arises from glial cells or their progenitor cells. As primary tumors, gliomas occur rather rarely with an estimated prevalence of 15 cases for 100 000 people (American Cancer Society, 2006). These anaplastic and malignant tumors result in a median of survival after diagnosis from 2 to 8 months, depending on the age of the patient (Curran et al., 1993; Scott and Mickle, 1987). One of the major problems in combating tumors is their ability to override the immune system. They are known to express a variety of immunosuppressive molecules like interleukin-10 (IL-10), prostaglandin E2 or transforming growth factor-β (TGFβ) (Wiendl et al., 2002; Wischhusen et al., 2002; Wrann et al., 1987). The secretion of these molecules leads to a local immunosuppression as well as to a systemic immunodeficiency (Platten et al., 2001).

The main treatment of glioma is surgical therapy, whereas the extent of the tumor resection closely correlates with the survival time of the patient (Jeremic et al., 2003; Keles and Berger, 2004; Soffietti et al., 1989). Nevertheless, brain surgery is not capable of removing all tumor cells. Thus, infiltrating tumor cells still reside in the healthy brain tissue and cause reformation of gliomas. Radiotherapy is another standard therapy for treatment of glioma.

But, in respect to long term survival, no effect of radiation alone is found in case of grade II gliomas (Karim et al., 2002).

In addition to these treatments, chemotherapy is commonly used. Chemotherapy aims at the impairment of cell division, which mostly affects fast-dividing cells including tumor cells. One of the biggest disadvantages of this therapy is the side effect caused by the effects on healthy dividing cells, which leads to hair loss and damage of the intestinal epithelium (Krauseneck and Muller, 1994).

Still, those therapies do not result in a satisfactory therapy of gliomas and there is need for new therapeutical approaches.

Experimental therapies are aiming at several mechanisms in tumor progression. On a molecular level, therapies aim at the cancer specific signal cascades and molecules to influence proliferation, invasion and angiogenesis (Adjei and Hidalgo, 2005; Krause et al., 2005). One of the most promising targets is TGFβ. Encouraging data of longer survival rates and slower tumor growth was obtained using a TGFβ antisense oligonucleotide for the treatment of human glioblastoma (Schlingensiepen et al., 2006). These findings were endowed by animal experiments and *in vitro* data (Jachimczak et al., 1996). The platelet derived growth factor receptor (PDGFR) is another possible candidate in molecular approaches of combating cancer. Upon inhibition of this receptor with imatinib, the tumor growth rate decreases and even complete remission can be observed in some patients (Dresemann, 2005).

Another approach in this field is the active specific immunotherapy which includes the *in vivo* induction of tumor-specific cytotoxic T lymphocytes. Upon induction, those cells are likely to be able to trigger apoptosis of tumor cells (Kagi et al., 1994).

During the recent years, focus has been cast on gene-based therapy in regard to malign gliomas. Mostly, adenoviral and retroviral vectors are used to deliver genes with therapeutic effects into the glioma site. The most promising candidates are oncolytic gene therapy and gene transfer of p53 or interferon-β (IFNβ) (Lang et al., 2003; Mineta et al., 1995; Yoshida and Tanaka, 2004). It was demonstrated that bone marrow derived cells express thymidine kinase of herpes simplex virus have a therapeutic effect on gliomas through bystander-mediated glioma cell killing after gancyclovir application (Miletic et al., 2007).

Microglia are known to be attracted by and to infiltrate the glioma site (Umemura et al., 2008). However, their phagocytic ability, antigen presentation and secretion of pro-inflammatory cytokines is suppressed by the tumor cells (Hanisch and Kettenmann, 2007; Komohara et al., 2008; Yang et al., 2010). These tumor-infiltrating microglial cells obtain an immunosuppressive phenotype and promote TGFβ production via an autocrine loop (Umemura et al., 2008). In addition to the immunosuppressive cytokine milieu provided by the glioma cells, this leads to T cell tolerance and inactivation of tumor targeted immune responses (Carpentier and Meng, 2006). Nevertheless, microglia are potent immune effector cells and are able to mediate the innate and boost adaptive immune responses. It is known that the classical activated microglial cell, in contrast to the alternatively activated microglia with an immunosuppressive phenotype, could phagocytose glioma cells and furthermore create a pro-inflammatory milieu (Mantovani et al., 2004). Additionally, chemokines from microglia are known to attract cytotoxic cells such as natural killer cells (Carter et al., 2007; Hughes et al., 2002; Napoli and Neumann, 2009), they yield a high potential for immunostimulatory therapy approach. Clearly, microglia are situated ideally within the CNS to confront migrating and resident tumor cells. Another feature of microglia is their ability to migrate from the blood stream into the CNS, which makes them an interesting

vehicle for therapeutic gene transfer into mature CNS tissue and the tumor residuum (Flugel et al., 2001).

Thus, the resolution of the immunosuppressive milieu by triggering a cytotoxic phenotype of microglia in the glioma site in combination with recruitment of cytotoxic leukocytes yields a promising approach. Especially microglial cells derived from embryonic stem cells appear suitable for therapy approaches, as they have unlimited proliferative potential and differentiated cells bear the potential to stimulate cytotoxicity towards glioma cells. Thus, iPSdM might therefore be a suitable and powerful tool for immune system-based or gene-transfer-based cell therapy of human glioma.

7. Regenerative approaches

Microglial cells are the immune effector cells which mediate immune responses in the CNS. They are known to play crucial roles in several neurodegenerative diseases such as multiple sclerosis (MS), AD or PD. Thus, they are an interesting target for regenerative therapies.

In MS, the immune system reacts with a destructive immune response against antigens of the CNS like myelin basic protein (MBP) or myelin oligodendrocyte glycoprotein (MOG), which leads to demyelination and axonal injury (Mattson and Taub, 2004). During disease course, scar-like plaques appear around the damaged axons, called lesions (Chari, 2007). Additional to the process of demyelination, inflammation is a hallmark of MS. This inflammation is mainly mediated by activated T cells, which invade the brain via the blood-brain-barrier and attack the myelin sheath of axons (Compston and Coles, 2002; Wucherpfennig and Strominger, 1995). This subsequently leads to stimulation of other immune cells like microglia and macrophages, which then secrete inflammatory cytokines (Cannella and Raine, 1995). This in turn leads to further recruitment of T cells, B cells and macrophages to the inflammation site (Steinman et al., 2002).

Until now, there is no known cure for MS, though some improvement in prevention of attacks and disability could be achieved through administration of immunosuppressants like interferons (Comi, 2009). Nevertheless, there is still need for other therapeutical approaches, especially in regard to neuroprotection and regeneration. Stem cell research has lead to new approaches in addressing neuroinflammatory disorders. Several cell types have been used for experimental therapies. Neural stem cells have been reported to migrate to inflammation sites in the CNS and to contribute to functional recovery and tissue repair (Pluchino et al., 2003). A similar beneficial effect was shown using bone marrow cells in experimental autoimmune encephalomyelitis (EAE), a mouse model for MS (Zappia et al., 2005).

It has been shown that microglia are able to pass the blood-brain-barrier and migrate into the brain under pathological conditions (Imai et al., 1997). In our group, we could show that ESdM like other bone marrow derived myeloid precursors migrate to lesion sites in EAE afflicted mice. Bone marrow myeloid precursors as putative progenitors of microglia have a beneficial impact after transduction with the microglial receptor triggering receptor expressed on myeloid cells-2 (TREM2) on the clinical course of EAE, on demyelination and axonal damage (Takahashi et al., 2007a). Furthermore, an anti-inflammatory milieu is established in the lesion sites due to the treatment (Takahashi et al., 2007a). Similar effects might be expected after transplantation of microglial precursor cells that are derived from ES cells and constitutively expressing TREM2.

As iPS-derived microglia principally can be generated from human iPS cells without oncogenic transformation and in high numbers, they might be a suitable and save vehicle

for neurotrophic factors to be released at inflammatory lesions in MS. In regard to AD, microglia can be seen as a possible therapeutic target, too. The pathological hallmarks of AD are cortical atrophy with accumulation of extracellular deposits of amyloid β (Aβ) in senile plaques and intracellular neurofibrillary tangles (NFT) of hyperphosphorylated tau in the cerebral cortex (De Strooper and Annaert, 2000). It is known that Aβ serves as a microglial activator and leads to production of various inflammatory mediators (Benveniste et al., 2001). On the other hand, a neuroprotective role of microglia was found in AD. Activated microglia migrate to Aβ plaques, where they release proteolytic enzymes like metalloproteinases that degrade Aβ (Qiu et al., 1997). Although it is unclear why microglia fail to completely clear the Aβ plaques by phagocytosis, at least they appear to restrict plaque growth (Bolmont et al., 2008). Nevertheless, it was reported that administration of exogenously applied microglia in a rat animal model of AD increases Aβ clearance (Takata et al., 2007). It has been shown by several groups that bone marrow-derived cells are capable of becoming brain macrophages, which then might play a major role in Aβ clearance (Malm et al., 2005; Simard and Rivest, 2004). Therefore, microglial cells may be a suitable tool for clinical application, if they are able to overcome the blood-brain-barrier. This problem could be addressed by irradiation (Ajami et al., 2007) or by intra-arterial injection (Imai et al., 1997), which both facilitates the entry of microglia into the brain. Otherwise, it would be possible to directly apply the iPS-derived microglia into the CNS by local transplantation. Thus, iPS-derived microglia might broaden the therapeutical approaches in AD, as they might be able to clear Aβ plaques and attenuate disease course.

8. Conclusion

Microglia are involved in a variety of neurodegenerative diseases. They are a promising target for new therapies. Their role as immune effector cells in the CNS might yield interesting perspectives for locally modulating the cytokine milieu or phagocytic properties to influence the disease course in a beneficial way. Glioma is the most common brain tumor which is not curable so far. Microglia were demonstrated to phagocytose glioma cells and attract cytotoxic cells to the glioma site. Therefore, they display a high potential for usage in therapy. In addition, microglia were shown to migrate from the blood stream into the CNS which makes them a suitable cellular vehicle for therapeutic gene transfer. Microglia overexpressing neurotrophic factors could contribute to the regeneration of lesioned brain tissue. Therefore, the generation of microglia from embryonic stem cells and iPS cells provides a new technology for combating cancer and for usage in regenerative medicine. Since iPS-derived microglia resemble human primary cells they can be further used in drug screening tests. It has been shown that very small sized particles such as nanoparticles could principally activate microglia. This leads to the production of pro-inflammatory cytokines and radicals by microglia which in turns promotes the pathology of neurodegenerative diseases. Therefore, iPS-derived microglia provide a new tool to evaluate the possible toxicity of very small-sized particles to the CNS.

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10. Conflict of interest

11. References

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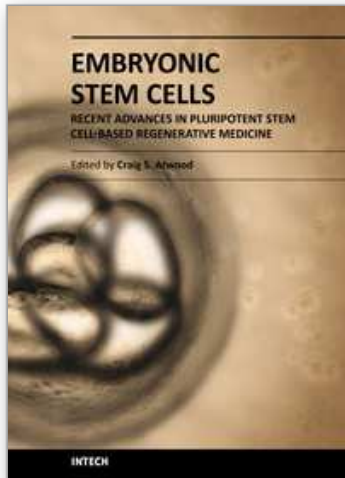
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