1. Introduction

1.1. Cancer in general

Cancer is a genetic disease which results from a multistep processes of multiple genetic alterations in a single cell (cancerogenesis; (Hanahan and Weinberg 2000). Pathological analyses of a number of organ sites reveal lesions that appear to represent the intermediate steps in a process through which cells evolve from normal via a series of premalignant states into invasive cancers (Foulds 1954). Hanahan and collegues suggest that the different cancer genotypes are a manifestation of six essential alterations in cell physiology and that they are governing malignant growth (Fig. 1.1.;(Hanahan and Weinberg 2000).

1.1.2. The hallmarks of cancer

1.1.2.1. Self-sufficiency in growth signals

Normal cells require mitogenic growth signals before they can move from a quiescent into the proliferative state. In contrast to cancer cells, where autonomy from external growth signals is achieved by different molecular strategies. Most evident mechanisms of autonomy from external growth factor stimulation are alterations of extracellular growth signals, of trancellular transducers of these signals, or of intracellular circuits that translate those signals into action (Fedi 1997).

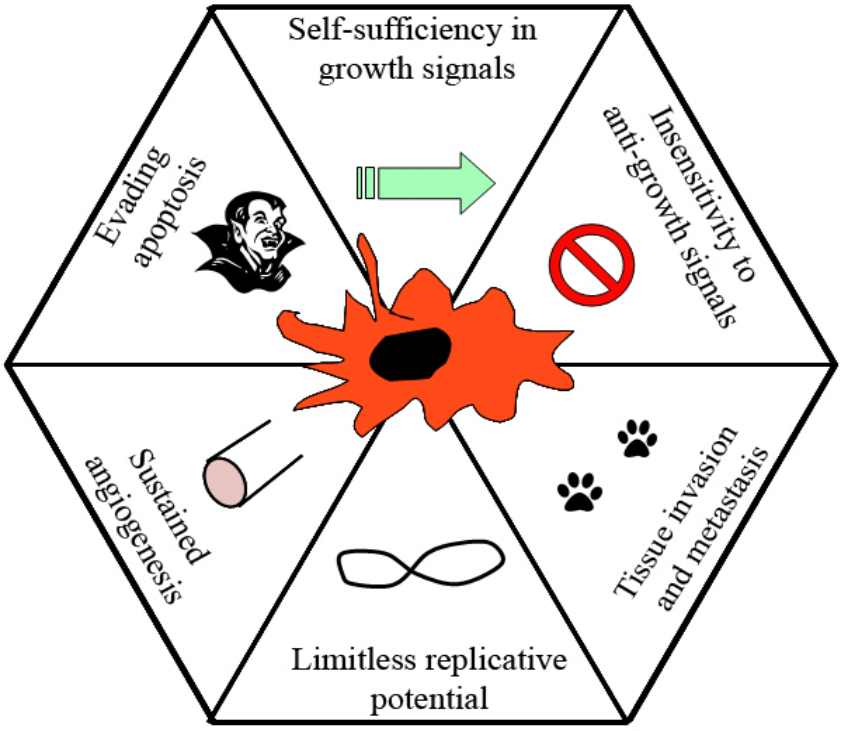


Fig. 1.1. Six essential alterations leading to cancer

1.1.2.2. Insensitivity to antigrowth signals

To maintain tissue homeostasis, the cell cycle in normal untransformed cells is strictly controlled by soluble factors or is restricted in cells which are embedded in ECM growth inhibitors, whereas the cancer cell evades these anti-proliferative signals. Disruption of the tumor suppressor pRB pathway is a major getaway for cancer cells to proceed in uncontrolled mitosis (Weinberg 1995).

1.1.2.3. Evading apoptosis

The characteristic of cancer cell populations to increase in number is not only determined by the strength of growth signals or insensitivity to antigrowth signals but also by the escape from the apoptotic program. Cancer cells get resistant to apoptosis by different mechanisms. The most common, detected in more than 50% of human cancers, is mutation or functional anomaly of the tumor suppressor p53 (Harris 1996). Additionaly, the AKT/PKB pathway, which transmits antiapoptotic survival signals, appears to be involved in avoiding apoptosis in a substantial fraction of human cancers, especially in gliomas (Evan and Littlewood 1998). Along with AKT

overactivation, one tumor suppressor that normally attenuates AKT activity- PTEN, is often mutated in gliomas, resulting in evasion of apoptosis (Merlo and Bettler 2004).

1.1.2.4. Limitless replicative potential

In normal cells, the number of cell generations is determined by the length of chromosomal ends, the telomeres. After each cell cycle, the telomeres get shortened and when all telomeres are spent the cell goes into senescence or undergoes apoptosis. But in human cancer the telomere maintenance is evident in 85-90% of all cells. The telomeres remain due to the upregulation of the enzyme telomerase, which adds hexanucleotide repeats onto the ends of telomeric DNA (Bryan and Cech 1999).

1.1.2.5. Sustained angiogenesis

All cells must reside within 100μm from capillary blood vessels in order to get oxygen and nutritients. Cancers as rapidly growing tissue, have developed a whole palette of mechanisms providing them with the new blood vessels. The most common mechanism is secretion of endothelial growth factors by tumor cells and by that directly stimulating neoangiogenesis in the tumor parenchyma (e.g. VEGF, PDGF and FGF (Hanahan and Folkman 1996).

1.1.2.6. Tissue invasion and metastasis

The characteristic of malignant cancers to spread out individual tumor cells into the parenchyma and into the rest of the body causes 90% of human cancer deaths (Sporn 1996). Change in cell-cell and cell-ECM interaction and overexpression of proteases are major determinants of invading cancer cells (Hanahan and Weinberg 2000). A well studied example of a change in cell-cell interaction is the loss of the cell adhesion molecule E-cadherin, which is ubiquitously expressed in ephitelial cells. Coupling between cells by E-cadherin signals for an antigrowth and anti-metastatic phenotype (Christofori and Semb 1999). E-cadherin function is lost in the majority of epithelial cancers, by mechanism that include mutational inactivation, transcriptional repression or proteolysis of the extracellular cadherin domain (Christofori and Semb 1999). A characteristic of most invading cancer cells is the increased proteolysis of

extracellular matrix which is mediated by an increased secretion of proteases or by a suppression of protease inhibitors. Additionaly, tumors are able to employ stromal or inflammatory cells to secrete active proteases or to provide the necessary intermediates for protease synthesis (Werb 1997; Le, Besson et al. 2003; Markovic, Glass et al. 2005).

1.1.3. Tumor stroma interaction

Initially, cancer research was predominated by investigations on the cancer cell alone, excluding the role of the surrounding stroma in cancerogenesis and during cancer progression (Hanahan and Weinberg 2000). The cancer stroma constituents in epithelial tumors are fibroblasts and cells of the innate and adoptive immune system (Bissell and Radisky 2001). These researchers were among the first to observe the tumor as a complex tissue, where cancer cells have subverted normal cell types into active collaborators, which promote neoplasia. Successful tumor cells are those that have acquired the ability to exploit their untransformed neighbours to release growth-stimulating signals (Skobe and Fusenig 1998) or to activate extra cellular matrix proteinases (Coussens and Werb 1996; Werb 1997). Moreover, several studies have demonstrated that stromal inflammation associated with tissue wounding or irradiation can induce cancer in non-demaged and non-irradiated epithelial cells (Sieweke and Bissell 1994). On the other side, Hill and colleagues recently demonstrated that cancer can induce surrounding stroma to lose p53 and by that become transformed (Hill, Song et al. 2005). Stromal cells can be induced to secrete matrix proteinases, thereby inducing cancerogenesis in the epithelium boosting existing tumors (Bissell and Radisky 2001).

1.2. The immune system of the brain

The central nervous system (CNS) is regarded as an immune privileged organ because of the presence of the blood-brain barrier (BBB), the lack of a fully organized lymphatic drainage system and the absence of expression of major histocompatibility complex (MHC) molecule class one (Wekerle 2002). The specialized endothelial BBB secludes the CNS parenchyma from the circulating blood and prevents most blood components from entering the tissue. Hence, the intact BBB is a barrier for

cells of the immune system, antibodies and other immunity-related molecules (Hickey 1991; Lassmann 1997). Under physiological circumstances, only activated T-lymphocytes are capable to pass the BBB and get into the brain (Lassmann 1997). They depart the brain if they do not encounter antigen presentation (Fabry, Raine et al. 1994; Lassmann 1997). In situations of acute or chronical brain damage or disease, activated leukocytes can in large number enter the brain even when the BBB is not opened (Brown 2001). The processes underlying these effect are still not clear. Next to blood derived activated leucocytes, the brain residents astrocytes, endothelial cells and microglia are also present at the site of brain injury. Only the microglia possess complete competence for immunological functions (Gehrmann, Matsumoto et al. 1995; Benveniste 1997). The microglia not only share the surface molecules with peripherial macrophages but are also capable of antigen presentation, phagocytosis and secretion of cytokines, chemokines and cytotoxins (Gehrmann, Banati et al. 1995). However, under pathological conditions, astrocytes are also able to secrete cytokines and chemokines (Asensio and Campbell 1999) and thus contribute to the immunological response of the brain.

1.2.1. The origin of microglia

Most researchers postulate microglial origin as mesodermal (Boya, Calvo et al. 1979; Ashwell 1990; Ashwell 1991; Streit 2005). Microglial precursors populate the CNS parenchyma early during embryonic development. The cells called fetal macrophages can be detected in the developing neuroectoderm at embryonic day 8 in rodents (Takahashi, Yamamura et al. 1989; Streit 2005). In 1932 Del-Rio Hortega discovered specific places in the embryonic brain where pial cells aggregated, which he called “fountains of microglia” (Del-Rio 1932). During late embryogenesis and perinatal period blood monocytes colonize the brain from the pia mater, evenly distribute throughout the brain and mature into microglia (Ling and Wong 1993). In perinatal stages they exhibit an amoeboid morphology with little cell processes indicating a rather active state phenotype with high proliferation rates (Farber and Kettenmann 2005). These cells are often referred to as microglial progenitors (Streit 2005). During the perinatal period microglia have one important role in CNS maturation, which is to regulate the specific neuronal fractions by differential induction of apoptosis (Marin-Teva, Dusart et al. 2004) and by phagocytosing the apoptotic neurons (Ling and

Wong 1993; Moore and Thanos 1996). Further, during brain development these amoeboid microglia differentiate into ramified microglia with a small cell body and a large number of fine and long membrane protrusions (Streit and Kincaid-Colton 1995; Streit 2005).

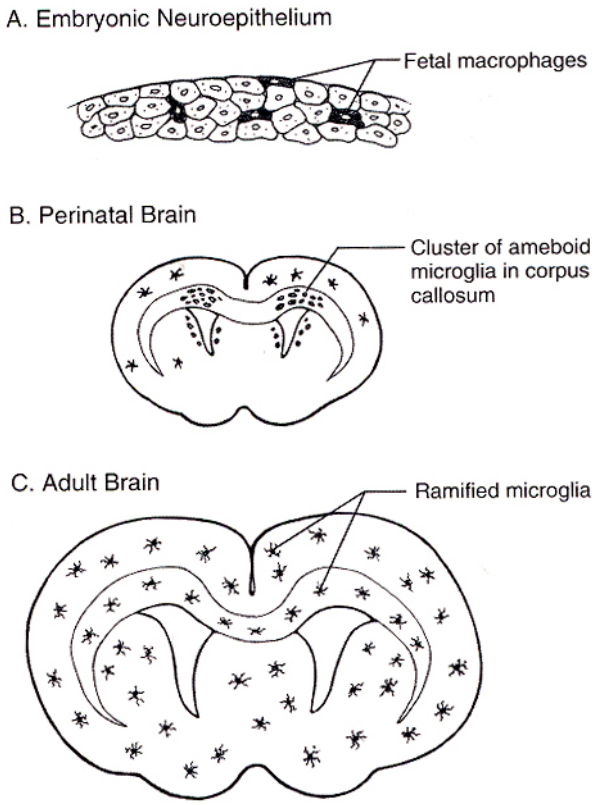


Fig. 1.2. Microglial developmental stages A. Fetal macrophages are found in the neuroectoderm of 8 days old embryonic brains. B. Groups of amoeboid microglia are found in the perinatal brain. C. Ramified microglia reside in the adult brain. Adopted from: Neuroglia, 2nd ed, Oxford University Press 2005.

1.2.2. The function of microglia

1.2.2.1. Resting or ramified microglia

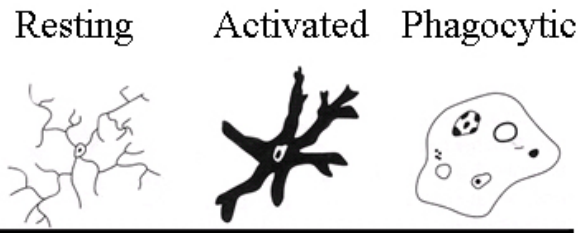
Microglia are present in all parts of the brain and represent 10 to 20% of all glial cells of the adult brain (Altman 1994). They are regularly distributed throughout the brain with neighbouring microglia not overlapping each others fine processes (Perry 1994; Nimmerjahn, Kirchhoff et al. 2005). Microglia are considered to be in the "resting" state in adult brain. Microglia in the "resting state" are characterized by a ramified morphology and the expression of certain cell surface antigens, like complement

receptor C3b (Wu, Wen et al. 1994; Kreutzberg 1996). The “resting state” suggests that the microglia are non-active and futile. However, this is not reflecting the authentic microglial profile (Raivich 2005). Lawson et al demonstrated that resting microglia proliferate at a low rate even in normal, not pathologically altered brain (Lawson, Perry et al. 1992). Moreover, the recent two-photon motion-picture study by Nimmerjahn (Nimmerjahn, Kirchhoff et al. 2005) demonstrated an active role of microglia in the mouse brain. Under physiological conditions, microglia very diligently move their ramified processes without moving their cell body. The Nimmerjahn study suggests that microglia survey every piece of the brain by moving their processes within short time periods. In the pathological state, microglia migrate within minutes, to the site of injury, travelling more than 100μm. At the site of the injury, they are rapidly shrinking their fine and long processes (ramifications) and enlarging their cell bodies.

1.2.2.2. Activated or amoeboid microglia

Activated microglia is a term used to describe microglial change in its physiological properties which usually appear under various pathological conditions of the brain such as viral or bacterial infection or CNS injury (Town, Nikolic et al. 2005). Microglial activation is followed by morphological changes (Streit, Graeber et al. 1988; Gehrmann, Banati et al. 1995). The ramifications are shortened, their number reduced and the cell body is enlarged, a stadium called amoeboid microglia (Kreutzberg 1996). Microglia activation increases gradually and can be further sub-characterized by increased cell motility, proliferation, phagocytosis and by the expression of new antigens (Streit, Graeber et al. 1988; Gehrmann, Matsumoto et al. 1995; Kreutzberg 1996; Streit, Walter et al. 1999; Streit 2005; Town, Nikolic et al. 2005) and also by the changed electrophysiological properties like established inward and outward rectifying potassium currents (Farber and Kettenmann 2005) and increased basal Ca^{2+} levels (Hoffmann, Kann et al. 2003). Activated microglia can release a diverse set of cytotoxic substances like reactive oxygen radicals, nitrate-monoxide (NO) and non-specific proteases which are important for the activation of cytokines by cytokine shedding (Banati and Kreutzberg 1993; Kreutzberg 1996; Zielasek and Hartung 1996). Cultured microglia are neither “resting” nor activated. They show rather amoeboid morphology with little ramified processes and possess

inward rectifying potassium current, whereas microglia in acute brain slices are characterised by a ramified morphology and the absence of potassium currents (Farber and Kettenmann 2005). Interestingly, when microglia is cultured with astrocyte conditioned medium they regain their ramified morphology (Eder 1998). This emphasizes their responsiveness to environmental cues and it points out that microglia research must be multisided and as close to in vivo settings to avoid artefacts of experimental approaches.



Proliferation	-/+	+	+
Griffonia simplicifolia B4-isolectin	+	+	+
Vimentin	-	+	+
Macrophage markers (ED1, ED2, OX-41)	-	-/+	-/+
CR3 complement receptor (OX-42)	+	+	+
MHC class I antigen	-	+	+
MHC class II antigen	-/+	+	+
CD4 antigen	-/+	+	+
CD8 antigen	-	-/+	+
Leukocyte common antigen	-	+	+

Table1.1. In vivo states of microglia biology and the associated phenotypic characteristics. Symbols: - absent; -/+ weak; + strong. Adopted from: Neuroglia, Kettenmann H and Ransom B, Oxford University Press 2005, pp63

1.3. Brain tumors

Gliomas are the most abundant primary tumors of the brain. They appear with an incidence of 5 in 100000 (Friese, Steinle et al. 2004) and with 30-40% of all brain tumors they present the largest group of these neoplasms in adults (Kleihues, Burger et al. 1993). Gliomas were classified in 1993 by the World Health Organisation (WHO) in four malignancy grades: Grade one (WHO I) are low malignant, pediatrical tumors; Grade two (WHO II) are low malignant oligodendrogliomas, diffuse-astrocytomas and ependymomas; Grade three (WHO III) are anaplastic-astrocytomas, oligodendrogliomas and ependymomas; Grade four (WHO IV) are most malignant glioblastomas (most common in adults) and highly malignant gliomas- like pineoblastomas and medulloblastomas (most common in children). It is still unknown wheather gliomas originate from immature astrocytes, mature astrocytes, or neuroectodermal stem cells located in the adult brain. Nevertheless, the pathological diagnosis relies on comparison of the gliomas with non-neoplastic mature glial cells (Weller 2003).

1.3.1. Role of microglia in brain tumors

1.3.1.1. Immune cell infiltration of intrinsic intracranial tumors

Immune cells, with the exception of microglia, are not infiltrating gliomas in large numbers. Strik et al reported in a study of 67 intracranial neoplasms from which 18 were glioblastoma multiforme (GBM) that 2% of the cells are tumor associated leukocytes (labelled for leukocyte marker LCA; (Strik, Stoll et al. 2004) whereas 15% are microglia (labelled for CD68). They describe the morphology of these tumor associated microglia as mostly amoeboid. To characterise CNS microglia Sedgwick et al. used flow cytometry by simultaneous CD11b/c and CD45 labelling (Sedgwick, Schwender et al. 1991). Microglia differ from macrophages in their low content of CD45. Therefore, microglia are identified as $CD45^{low}$ $CD11b/c^{high}$ cells and macrophages as $CD45^{high}$ $CD11b/c^{high.}$. Using this method Badie et al. showed that microglia were detected in high number in the brain tissue surrounding the tumors (Badie and Schartner 2000; Badie and Schartner 2001) in contrast to macrophages which were only found inside the tumors. Along that line, Roggendorf et al reported in a neuropathological study that a vast number of amoeboid microglia is located in

peripherial tumor areas, where the tumor shows diffuse infiltration into surrounding brain tissue (Roggendorf, Strupp et al. 1996).

The exact resource of microglia in brain tumors remains unclear. A variety of growth factors and chemokines can be released directly from the tumor cells or as a result of local tissue injury due to tumor growth and metastases (Badie and Schartner 2001). This can result in the recruitment of microglia and macrophages from two main sources: resident brain microglia or perivascular macrophages that can become activated and migrate toward brain tumors (Watters, Schartner et al. 2005). Furthermore, CNS parenchyma can be populated by trafficking hematopoetic cells such as monocytes that can assume typical microglial morphology upon entry into the CNS (Flugel, Bradl et al. 2001). Regardless of their origin, these microglia and macrophages can continuously infiltrate brain tumors and influence tumor growth (Villeneuve, Tremblay et al. 2005).

1.3.1.2. Microglia-glioma cross talk

A number of studies demonstrate the intense communication between glioma cells and microglia. Microglia are attracted by several glioma secreting chemoattractants: Monocyte chemoattractant protein-1 (MCP-1; (Prat, Baron et al. 2000), acts on microglia receptor CCR2 (Galasso, Stegman et al. 2000), colony stimulating factor-1 (Papavasiliou, Mehler et al. 1997), granulocyte colony-stimulating factor (G-CSF;(Stan, Walter et al. 1994) and hepatocyte growth factor/scatter factor (HGF/SF) acts on microglia receptor HGF/SF –Met (Koochekpour, Jeffers et al. 1997). The further interaction of microglia and gliomas is complex and largely uninvestigated. It is presently debated whether the presence of microglia in and around tumors is an attempt by the immune response to combat the tumor, or whether microglia are recruited by the tumor to promote tumor growth and proliferation. On the one hand, microglia may act as an anti-tumor response by releasing anti-tumor cytokines like TNFα, or behave as an antigen presenting cell (APC) expressing MHC II and B7.1 and B7.2 molecules. However, there is little evidence that the release of cytotoxic cytokines is the main action of tumor infiltrating microglia. Furthermore, microglia are weak APC in glioma due to the abundance of IL-10 in the tumor (Stan, Walter et al. 1994; Wagner, Czub et al. 1999). On the other hand, it is possible that microglia promote glioma proliferation and invasion via pro tumor secreted cytokines.

1.3.2. Glioma cell evasion of the immune response

Patients with malignant gliomas show decreased cellular immunity (as assessed by delayed-type cutaneous reactions) and a reduced number of circulating T cells due to selective depletion of T helper cells (Brooks, Netsky et al. 1972; Brooks, Roszman et al. 1977; Mahaley, Brooks et al. 1977; Mahaley, Gentry et al. 1977). Characteristic for glioblastomas is that they do not systemicly metastasize (Schweitzer, Vince et al. 2001; Stark, Nabavi et al. 2005). Only in very rare cases, several cases of extraneural metastases following organ transplantation have been reported, showing tumor growth in transplanted organs under immunosuppression. After withdrawal of immunosuppressive drugs extraneural glioma cells were eradicated in several cases, suggesting an immunological control of glioma cells outside the CNS, while the CNS milieu supports tumor growth and inhibits immune responses (Schweitzer, Vince et al. 2001). Hao et al quantitatively analyzed the expression of 53 cytokines and cytokine receptors in human gliomas and glioma cell lines (Hao, Parney et al. 2002). The results of this quantitative study indicate that the strongly immunosuppressive cytokine response greatly predominates in both human solid tumors and in glioma cell lines. For example, the cytokines interleukin (IL)-6, leukemia inhibitory factor (LIF), oncostatin-M (OSM), and TGF ß and their receptors were strongly expressed in nearly all glioblastomas and cell lines tested, whereas the proinflamatory cytokines interferon (IFN)γ, tumor necrosis factor (TNF)α, and the IL-2 and IL-12 family members and their receptors were virtually absent in both the tumors and cell lines (Hao, Parney et al. 2002). Others have observed similar expression patterns of immunosuppressive cytokine expression in gliomas. All IL-6 type cytokines (IL-1, IL-11, CNTF, CT-1, LIF, and OSM) have been detected at the protein and mRNA levels in glioma cell lines (Murphy, Bitting et al. 1995; Goswami, Gupta et al. 1998; Halfter, Kremerskothen et al. 1998; Halfter, Lotfi et al. 1998; Hao, Parney et al. 2002) as have been all members of the TGF ß cytokine family (Constam, Philipp et al. 1992; Olofsson, Miyazono et al. 1992; Hao, Parney et al. 2002). TGF ß for example has been shown to inhibit the proliferation of microglia as well as their production of cytokines in vitro (Suzumura, Sawada et al. 1993), whereas the cytokines IL-6 and IL-10 have been postulated to promote glioma cell proliferation (Huettner, Czub et al. 1997; Goswami, Gupta et al. 1998). The immunosuppressive cytokine IL-10 not only promotes glioma cell proliferation, but it also enhances their ability to migrate in vitro (Huettner, Czub et al. 1997), further supporting the importance of microglia–glioma cross talk. Immunosuppressive glioma effects on leukocyte apoptosis have also been

observed. Badie and colleagues demonstrated previously that leukocyte infiltration into subcutaneously (SC)-propagated GL261 mouse gliomas was much greater than into intracranial (IC)-propagated tumors, again suggesting that in the microenvironment of the brain plays an immuno-suppressive role (Badie, Schartner et al. 2001). Furthermore, they postulate that the threefold increased expression of FasL by monocytes in IC gliomas compared to SC gliomas is pro-apoptotic for circulating, glioma infiltrating leukocytes.

1.4. Matrix metalloproteinases

Extracellular proteases are crucial regulators of cell function. The family of matrix metalloproteinases (MMPs) has been described in the context of extracellular matrix (ECM) remodelling, which occurs throughout life in diverse processes that range from tissue morphogenesis to wound healing. Recent evidence has implicated MMPs in the regulation of other functions, including survival, angiogenesis, inflammation and signalling (Chakraborti, Mandal et al. 2003). There are at least 25 members of the MMP family and, collectively, these proteases can degrade all constituents of the ECM. They have been classified into different classes based on their substrate specializations (collagenases, gelatinases, stromelysins and others), their structure and on their transmembrane localizations (Table1.2.). Some MMP family members are covalently linked to the cell membrane-membrane type MMPs (MT-MMP) and they are specialized for localized and controlled proteolysis (Badie, Schartner et al. 2001). The other family members are secreted into the exracellular space as inactive zymogens (proMMPs). Their activation requires proteolytic removal of the propeptide domain. Once secreted and activated, they are inhibited by a family of endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs; (Woessner 1991; Greene, Wang et al. 1996). The balance between the levels of activated MMP and free inhibitors determines the overall MMP activity (Mohanam, Wang et al. 1995). As a result of their potent proteolytic activity, abnormal MMP function can also lead to pathological conditions (Hotary, Allen et al. 2003).

Matrix metalloproteinase family members

Member	Name	*M* latent/ active (kDa)	Furin activation site	Collagen substrates	Pro-MMP substrates	Other matrix substrates*
MMP1	Collagenase 1	55/45		I, II, III, VII, VIII, X	2, 9	Agg, Gel, PG
MMP2	Gelatinase A	72/66		I, III, IV, V, VII, X, XI, XIV	1, 9, 13	Agg, EL, FN, Gel, LN, PG, VN
MMP3	Stromelysin 1	57/45		III, IV, IX, X, XI	1, 7, 8, 9, 13	Agg, EL, FN, Gel, LN, PG, VN
MMP7	Matrilysin	28/19		IV, X	1, 2, 9	Agg, Casein, EL, FN, Gel, LN, PG, VN
MMP8	Collagenase 2	75/58		I, II, III, V, VII, VIII, X		Agg, EL, FN, Gel, LN,
MMP9	Gelatinase B	92/86		IV, V, VII, X, XIV		Agg, EL, FN, Gel, PG, VN
MMP10	Stromelysin 2	57/44		III, IV, V, IX, X	1, 8	Agg, EL, FN, Gel, LN, PG
MMP11	Stromelysin 3	51/44	Yes			
MMP12	Metalloelastase	54/45/22[‡]		IV		Casein, EL, FN, Gel, LN, PG, VN
MMP13	Collagenase 3	60/48		I, II, III, IV, VII, IX, X, XIV	9	Agg, FN, Gel
MMP14	MT1-MMP	66/56	Yes	I, II, III	2, 13	Agg, EL, FN, Gel, LN,
MMP15	MT2-MMP	72/60	Yes		2	Agg, FN, Gel, LN
MMP16	MT3-MMP	64/52	Yes	III	2	Gel, FN
MMP17	MT4-MMP	57/53	Yes			Fibrinogen/fibrin
MMP18	Collagenase 4	70/53		I		
MMP19	RASI 1	54/45		IV		Gel, FN, LN
MMP20	Enamelysin	54/22				Amelogenin
MMP21	*Xenopus* MMP	70/53	Yes			
MMP22	Chick embryo MMP	51/42				Casein, Gel
MMP23			Yes			
MMP24	MT5-MMP		Yes		2	Gel
MMP25	MT6-MMP		Yes	IV		Gel, FN
MMP26	Matrilysin 2/endometase	28/19				Gel
MMP27	Human MMP22[§]					
MMP28	Epilysin	56/45	Yes			Casein

Table1.2. Matrix Metalloproteinase family members
adopted from *Nature Reviews Neuroscience* 2, 502-511 (2001)

1.4.1. Regulation of MMPs

MMP activity is regulated by the gene expression, pro-enzyme activation and inhibition of active enzymes by their specific inhibitors. The expression of MMPs is induced by cytokines, growth factors, physical stress, oncogenic transformation, and cell-matrix and cell-cell interactions (Rao 2003). The literature is aboundant with examples of normal MMP gene expression which may be cell type-specific, tissue-specific and even stage-specific as well as constitutive and inducible (Matrisian 1994).

Mitogen-activated protein kinases (MAPKs) phosphorylate specific serines and threonines of target substrates and by that regulate many important cellular activities including gene expression, mitosis, movement; metabolism and programmed death. There are three well characterized subfamilies of MAPKs (Johnson and Lapadat 2002). These include the extracellular signal-regulated kinases, ERK1 and ERK2; the c-Jun amino-terminal kinases, JNK1, JNK2 and JNK3; and the four p38 enzymes, p38alpha, p38beta, p38gamma and p38delta. They have been studied extensively to define their roles in the regulation of MMP gene expression. Most notably, the

expression of specific MMP is tightly regulated by a distinct MAPK pathway and it is mostly tissue and cell specific (Reuben and Cheung 2006). Along with that, the smooth regulation of active MMP release is obtained by controlled cleavage of pro-enzyme parts by the other extracellular proteases (Le, Besson et al. 2003; Itoh and Seiki 2004).

1.4.1.2. Extracellular proMMP-2 activation cascade

Since the MMP-2 secretion and activation plays important role in glioma pathogenesis (Lampert, Machein et al. 1998), the activation cascade of proMMP-2 in extracellular space is described (Fig. 1.1.).

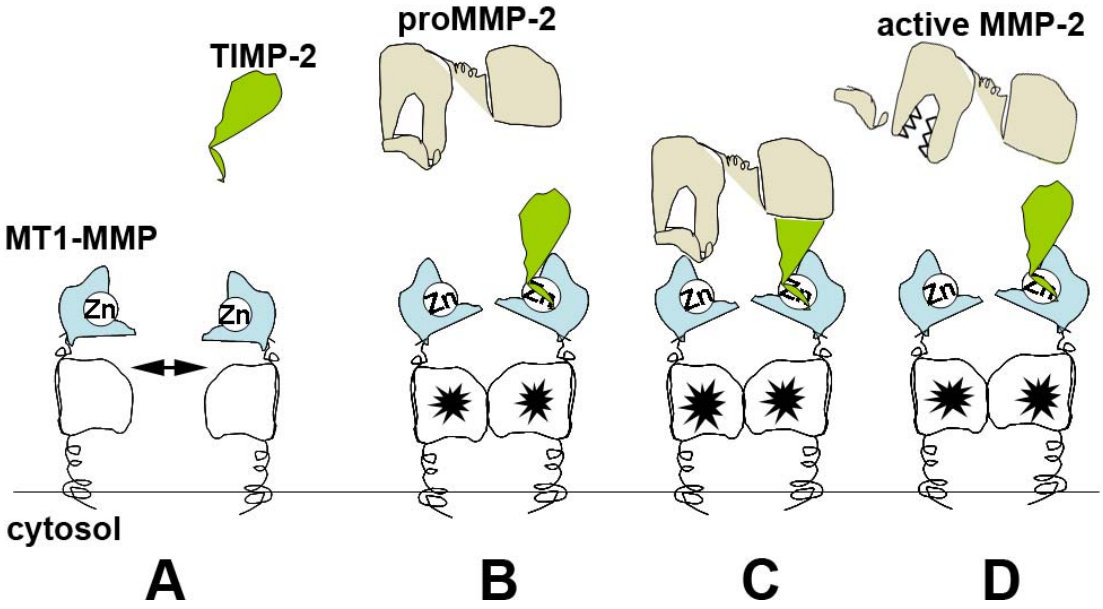


Fig. 1.3. Activation cascade of MMP-2. At least two MT1-MMP molecules must be associated (A), in order to dock one TIMP-2 molecule (B) which in turn binds one proMMP-2 molecule (C). Finally, the active MT1-MMP complex cleaves pro-MMP-2 and releases active MMP-2 (D).

To activate proMMP-2 on the cell surface, at least two molecules of MT1-MMP must be in close proximity. This is achieved by the formation of a homophilic MT1-MMP complex (Fig. 1A). One of the enzymes in the complex binds to TIMP-2 to form an enzyme-inhibitor complex (Fig. 1B). The exposed TIMP-2 C-terminal domain then binds to proMMP-2, positioning this pro-enzyme optimally for activation by the second active MT1-MMP in the complex (Fig. 1C). Then this active MT1-MMP cleaves the pro enzyme part from proMMP-2 (Fig. 1D). Finally, active MMP-2 can be released from TIMP-2 (Itoh and Seiki 2004).

1.5. The MMPs and cancer

The most widely studied pathological process that involves MMPs is cancer invasion and metastasis (Yong, Power et al. 2001). The tumor cell is thought to use MMPs to overcome multiple structural barriers and establish a new focus of growth at a distant site from the primary tumour mass. In addition, other features of tumor evolution, including survival, growth, and angiogenesis, also may be dependant on MMPs (Chambers and Matrisian 1997).

1.5.1. The role of metalloproteinases in brain tumors

A positive correlation between the glioma malignancy and MMP levels has been documented in brain tumors (Yamamoto, Mohanam et al. 1996). Lampert et al analysed the mRNA expression level of 15 MMPs and three TIMPs in gliomas, medulloblastomas and normal brain tissue (Lampert, Machein et al. 1998). They found that MMP-2;-9;-14, and 15 and TIMP-1 are overexpressed. They observed a significant increase of the proteinases and proteinase inhibitors in glioblastomas compared to low-grade astrocytomas, anaplastic astrocytomas and normal brain. Moreover, study from Forsyth (Forsyth, Wong et al. 1999) highlights three MMPs: the MMP-2, MMP-9 and MT1-MMP as the most predominantly expressed MMPs in malignant gliomas. The MMP-2 and 9 have been connected closely with the tumor invasiveness because of its potent ability to degrade the type IV collagen present in basement membrane that surrounds blood vessels.

1.6. Aim of the study

1. To develop an in vitro glioma invasion model, which resembles the complex cellular and extracellular brain tissue environment and which allows selective microglia depletion.
2. To quantify the net effect of microglia presence/absence on glioma invasion in organotypical brain slice cultures model.
3. To reveal the most dominant mechanisms underlining microglia-glioma interaction.
4. To indentify soluble microglia or glioma released factors which are promoting glioma invasiveness.
5. To propose a therapeutical approach on the basis of the discovered microglia-glioma cross-talk.