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Aquaporin, Midkine and Glioblastoma

Mine Ergüven

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1. Introduction

1.1. Glioblastoma

Glioblastoma (GBM) with their invasive and aggressive nature, are the most common primary brain tumours. GBM accounts for about 60% of all gliomas and 12–15% of all brain tumors, and it is per se the most frequent primary brain tumor [1,2]. Although advances in therapies, clinicians and researchers fail to arrive at overcoming poor prognosis with a median survival of only one year from the time of diagnosis. In Europe and North America, the incidence is three new cases per 100.000 inhabitants per year [3]. Although GBM can manifest itself at any age, it preferentially occurs in adults, with a wide peak age of incidence between 45 and 70 years [4].

GBMs arised from glial cells which are the building-block cells of the connective and supportive, tissues in the central nervous system. Diffuse gliomas defined as astrocytomas, oligo-dendrogliomas, and oligoastrocytomas are the common gliomas which infiltrate throughout the brain parenchyma. They are graded on a World Health Organization (WHO) classification system scale of I to IV according to their degree of malignancy based on different histological features and genetic alterations. Grade I tumors are pilocytic astrocytomas and they are benign and can be cured if they can be surgically resected; grade II tumors are low grade astrocytomas (LGAs) which are incurable with surgery because of their early diffuse infiltration of the surrounding brain, and long treatment regimens are needed to treat this disease completely; grade III tumors are anaplastic astrocytomas and they have increased anaplasia and proliferate over grade IV tumors and are more rapidly fatal; grade IV tumors are GBMs which possess advanced features of malignancy, and are resistant to radio/chemotherapy [5].

Important characteristics of GBMs are aberrant cellular proliferation, diffuse infiltration, prospensity for necrosis, robust angiogenesis, high resistance to apoptosis, and genomic instability. The intratumoral heterogenity combined with a putative cancer stem cell

(CSC) subpopulation and incomplete atlas of epigenetic lesions are the reasons of poor prognosis/high tumoral resistance against chemotherapeutics and recurrence [1,2,5-8]. Studies showing crosstalks between genetics and epigenetics in GBM are highlighted to solve mystery [5-7]. There are two types of GBM: Type 1 GBM typically shows inactivation of the TP53 tumor suppressor gene but no amplification of the EGFR oncogene. Mutations of p53, mostly associated with loss of heterozygosity (LOH) in the 17p chromosome region, can be observed in GBM originating from a less malignant glioma precursor. TP53 inactivation does not occur together with amplification of the EGFR oncogene, which is only identified in GBM without TP53 mutation More than 70% of malignant gliomas show a deregulated TP53 pathway not only by mutation of TP53 but also amplification of MDM2, homozygous deletion/mutation, or promoter hypermethylation-mediated silencing of CDKN2A. Type 2 GBM shows overexpression or amplification of the EGFR without mutations of TP53, and it appears de novo, that is, in patients without a less malignant precursor neoplasm such as grade II or III astrocytoma [6,7].

Two independent GBM pathways were also identified [9]. Moreover, epidermal growth factor receptor (EGFR) amplification is almost always consistent with LOH in chromosome region 10q [16]. The tumor suppressor gene Phosphatase and tensin homolog (PTEN), mapping the 10q23 region, is mutated in approximately 30% of type 2 GBM [6,10]. Mutations in this gene have been described only in malignant gliomas and are rarely associated with p53 mutations. Other frequent mutations in type 2 GBM affect the cyclin dependent kinase (CDK) cell-cycle-regulator genes. Amplification of CDK4 and CDK6 was observed in 15% of type 2 GBMs [6,7]. Mutations of the cell-cycle-regulator genes CDKN2A/CDKN2B have been observed in 40% of all GBM. Moreover, a functional loss of expression of the CDKN2A gene by promoter hypermethylation was found in 15% of GBM [6,7]. Mutations of the Isocitrate dehydrogenase 1 (IDH1) gene have been frequently observed in those GBM progressing from a less malignant precursor lesion, that is in type 1 GBM, mostly of them affecting young patients. Interestingly, these IDH1 mutations were associated with a better outcome [6]. In addition to type 1 and type 2 GBMs, there are other forms, whose molecular profiles do not identify them as belonging to either of the two classic pathways [6,7].

The methylation signature of gliomas is also rather associated with tumor lineage and malignancy grade. Thus, astrocytomas grades WHO II and III and GBM grade IV show different methylation status of several genes. Even though, primary and secondary GBMs were found to differ concerning methylation of genes which was associated with decreased mRNA levels. In this context, methylation of methyl guanine methyl transferase (MGMT) is more frequently observed in 75% of secondary GBM than in primary GBMs (36%). Moreover, MGMT methylation has been observed to be associated with TP53 mutations in secondary GBMs. Cellular pathways deregulated in gliomas and associated epigenetic events through promoter hypermethylation, CpGs hypomethylation, and histone alterations leading to modified chromatin states are Ras signaling (RASSF1A, RRP22, DIRAS3), Cell migration and adherence (NECL1, E-cadherin, SLIT2, EMP3, TIMP3), Wnt signaling (WIF1, FZD9, IGFBP-3, SFRP family, PEG3), Tyrosine kinase pathways (KIT, SYK, c-ROS),Transcription factors (SOX2, KLF4, GATA 6, ATOH1), Homeobox genes (HOXA 9, HOXA10, HOXA11), Sonic hedgehog

signaling (PTCH1, Cyclin D2, Plakoglobin, PAX6, NKX2.2), Notch signaling (NEURL1, HES1, HEY1), bone morphogenic protein (BMP) developmental pathway (BMPR1B), Hypermutator pathways (hMLH1, hPMS2, MGMT,WRN), Apoptosis (TMS1, DAPK1, CASP8, DR4, DR5), TP53/cell cycle (HIC-1, CDKN2A, RB1, p16INK4a), MicroRNAs (miR-124a, miR-21, miR-7, miR-137, miR12) [6,7,11].

MGMT can be given for instance for these pathways MGMT, which has been observed to be hypermethylated in low-grade gliomas (grade II) further evolving to gliomas grade III and GBM [6,7]. Furthermore, this biomarker allows neurooncologists to predict patient's response to current chemotherapy with temozolomide [12]. Second instance can be microsatellite instability (MSI) which was observed to be more frequent in those GBMs evolving from less malignant gliomas grade II or III, which typically display TP53 mutations without EGFR amplification, as well as in relapse GBM [5,6]. Third instance can be given as epigenetic inactivation ofone of the apoptosis-related genes (TMS1/ASC and DAPK1, WIF-1, SFRP1 and CASP8) as proapoptotic gene CASP8 which is an epigenetic silenced during progression of primary-to-recurrent GBM. GBM can change its epigenetic profile quickly, therefore its adaptation (heterogeny) to novel therapies is big obstacle [6,7,11].

Because of incomplete atlas of genetic and epigenetic pathways, gene therapies and chemotherapies have limited efficacy and they are under investigation [6,7,13,14]. Researchers and clinicians are trying to fight this monster following products of these genes [15-17]. Many "Trojan horse" approaches, based on potential applications in the pharmacological therapy of GBMs which blood-brain barrier (BBB) represents an obstacle are being proposed day by day [18,19]. The passage of drugs across the BBB limits the efficacy of chemotherapy in brain tumors [18-21]. Many anti-neoplastic drugs evaluated as "magic bullets" that is effective against glioblastoma in vitro, has poor efficacy in vivo or has both efficacy in vitro and in vivo, has poor efficacy in clinic because it is extruded by P-glycoprotein (Pgp/ABCB1), multidrug resistance-related proteins (MRPs) and breast cancer resistance protein (BCRP/ABCG2) of BBB cells [20,21]. Although these proteins are commonly studied in order to overcome drug resistance for several decades, GBM attack with other weapons in order to win this war and it continues to surprise researchers and clinicians [20,21]. Other weapons of GBM can be defined as aquaporins (AQPs) [22,23].

2. AQPs

AQPs, water channels, have been proposed as novel targets in cancer and oedema and are associated with a surprising array of important processes in the brain and body, such as angiogenesis, cell migration, development and neuropathological diseases. In both cancer and brain oedema, current therapies are limited and new pharmacological approaches focused on AQPs offer exciting potential for clinical advances [23]. The expression of six isoforms of AQP protein (AQP1, 3, 4, 5, 8, 9) has been reported in the glial cells [in astrocytes (AQP1, 3, 4, 5, 8, 9), oligodendrocytes (AQP8), tanycytes (AQP9) and ependymal cells (AQP1, 4, 9) [24]. As astrocytes are the most numerous glial cell type and account for one third of brain mass [25]

and they are involved in the maintenance of the blood–brain barrier (BBB), and as the GBM is the most malignant form astrocytic brain tumor [1,2], we focused on the AQPs on the astrocytes related to GBM. Previous reports showed that AQPs 1, 4, and 9 have significant roles in the pathogenesis of malignant brain tumours [24].

3. AQP1

AQP1 plays an important role in water transport in expressed in various organs and cells (microvascular endothelial cells, kidney, central nervous system, eye, lacrimal and salivary glands, respiratory apparatus, gastrointestinal tract, hepatobiliary compartments, female and male reproductive system, inner ear, skin) [26]. Previous reports sowed that brain astrocytes express AQP1 under pathologic conditions as the early stage of Alzheimer disease, subarachnoid hemorrhage, cerebral infarction [27-29]. Monzani and coworkers showed a role for AQPs in facilitating cell migration at the first time for AQP1 in human endothelial and melanoma cell lines in vivo [30].

Hypoxia stimulates astrocytic migration it is possible that hypoxic conditions after spinal cord injury (SCI) trigger AQP1 synthesis in astrocytes, as an attempt of injured spinal cords to facilitate astrocytic migration to the lesion site [31,32]. Hypoxic conditions may contribute to chronic accumulation of water within neurons and cytotoxic edema in chronically injured spinal cords [33]. AQP1 expression in spinal cord may have a role in axonal remodeling and plasticity, necessary for normal sensory processing [34]. Abreu-Rodríguez and coworkers showed that HIF-1α participates in the hypoxic induction of AQP1 in 9L glioma cells. They also demonstrated that the activation of AQP1 promoter by hypoxia is complex and multifactorial and suggested that in addition to HIF-1α other transcription factors might contribute to this regulatory process [35].

GBMs express increased aquaporin AQP1. AQPs may contribute to edema, cell motility, and shuttling of H_2O and H^+ from intracellular to extracellular space [36]. In comparison to normal brain, GBMs have different vascular structures and metabolic changes. GBM cells make higher aerobic glycolysis under hypoxia than under normoxia leading to invasion of cancer cell [37,38].

4. AQP4

AQP4, the most important water channel in the brain, is found in supporting cells as astrocytes (astrocyte endfeet abutting microvessels), ependyma and its also found in retina [39, 40]. AQP4 expression is polarized in astrocytes and AQP4 redistributes throughout the astrocyte cell membrane, suggesting that endothelial cells signal astrocytes to polarize AQP4 expression in the cell membrane [41]. Previous study showed that AQP4 is involved in the formation and resolution of brain and spinal cord edemas. In the absence of AQP4, brain edema is decreased and neurologic improvement following ischemic brain injury is increased [42].

AQP4 expression is commonly up-regulated in astrocytes associated with brain edema [42]. It was showed that an up-regulation and redistribution of AQP4 accompanied by a loss of its polarized expression pattern and so the evidence for a role of it in vasogenic edema formation in GBM [43, 44]. Altered expression levels of AQP4 and redistribution of the protein throughout the membranes of cells are found in GBM and this leads to development of the oedema often found surrounding the tumour mass. AQP4 also facilitates the elimination of excess brain water. Excess water is eliminated primarily through the glia limiting membranes into the CSF that vasogenic edema fluid is eliminated by an AQP4-dependent route. AQP4 pumps out excess fluid assembled in the brain parenchyma after BBB disruption [43,44]. Wang and coworkers suggested that HIF-1α plays a role in brain edema formation and BBB disruption via a molecular signaling pathway involving AQP4 and matrix metalloproteinase 9 (MMP-9) [45]. HIF-1α binds the promoter of AQP4 resulting in the increase in its' expression [46].

It was shown that in human immunodeficiency virus (HIV) infected patients, AQP4 expression was increased indicating the role of AQP4 in a protective and/or maladaptive response to CNS inflammation [47,48].

Recent study have found changes in astroglia Kir and AQP4 water channels in temporal lobe epilepsy specimens [49]. Dysregulation of AQP4 also occurs in hippocampal sclerosis and cortical dysplasia in patients with refractory partial epilepsy [50]. These are clue for both AQP4 and Kir4.1 participate in clearance of K$^+$ following neural activity. Othe report suggested that AQP4 and Kir4.1 may also act in concert in K$^+$ and H$_2$O regulation [51]. K$^+$ re-uptake into glial cells might be AQP4-dependent, as water influx coupled to K$^+$ influx is thought to underlie activity-induced glial cell swelling [52]. Further studies are required to clarify the expression and functional interaction of AQP4 and Kir4.1 in the hippocampus and their changes during epileptogenesis.

Recent reports have also suggested a role of AQP4 for neuroglial activation in autism and more studies are also needed as epileptogenesis to confirm its spesific role in autism [53].

AQP4 is highly expressed in the basolateral membrane of the ependyma and glia limitans. This meaning of this distribution feature can be evaluated that AQP4 provides a highly efficient pathway to transport the redundant water from parenchyma to ventricle system and subarachnoid space [42]. The highly polarized expression of AQP4 may be involved in the structural and functional integrity of the ependyma maintance [42,54,55]. AQP4 is highly related with the gap junction protein connexin43 (Cx43), which is the main gap-junction protein in astrocytes as well as ependymal cells [53,55].

Previous report showed that reactive microglial cells expresses AQP4 mRNA and protein in *in vivo* [56]. All cells which are expressing AQP4 in microglial cells may represent a molecular adaptation to maintain ion water homeostasis in the injured brain. Activated microglia is important in the clearance of K+ and restoration of osmotic equilibrium in absence of astrocytes. It is well known that glial cells play an important role in regulating the homeostasis to ensure an appropriate neuronal environment [24,56,57]. AQP4 seems to play an essential role because of the possible role of astrocytes in pomping out excess K$^+$ around active neuron [24,56,57].

5. AQP9

AQP9 transports glycerol, mannitol and urea. It was firstly found in human leukocytes, and it is also expressed in liver, testis, and brain [58,59]. In the brain, AQP9 is expressed in tanycytes (they possess no cilia). The tanycytes are found in circumventricular organs of the third ventricle lacking a BBB [60, 61]. More studies are needed to confirm AQP9 expression in the subset of ciliated ependymal cells [60]. AQP9 is also expressed in astrocytes and spinal cord of the glia limitans and white matter tracts. Its expression is throughout the astrocyte cell bodies and processes in the brain [62]. AQP9 may play a role in extracellular water homeostasis/oedema and it also helps glycerol and monocarboxylate diffusion [63].

In addition, it is proposed that AQP9 plays a role in clearing lactate from the extracellular space in pathological ischemic conditions. Most glioma cells throughout the tumour revealed a strong AQP9 expression across the whole surface of the cells in human GBM. AQP9 expression is increased in all grades of human astrocytic tumours and this expression is increased from low-grade tumours to high-grade tumours [64]. The increase of AQP9 expression is essential for the clearance of glycerol and lactate from the extracellular space at the glioma-associated lactic acidosis [65]. AQP9 expression may account for GBM resistance to hypoxic and ischemic situations, by facilitating clearance of lactate and glycerol resulting from hypoxia and cellular damage, respectively [66-69]. HIF-1α binds AQP4 promoter, consequently it increases the expression of AQP4 [70].

It might, therefore, play a role in both the energy metabolism of normal brain tissue and provide increased tolerance for hypoxia under pathological conditions. AQP9 may play an important role in the malignant progression of brain tumours and it can be used as a biomarker for molecular diagnosis and as a new target for gene therapy.

6. AQPs 1,4,9 in stem cells

In the study of Fussdal and coworkers at biopsies from GBMs, they analyzed the expression of AQPs 1, 4, and 9 in isolated tumour stem cells grown in a tumoursphere assay and analyzed the progenitor and differentiated cells from these cultures. They compared these expressions to the situation in normal rat brain, its stem cells, and differentiated cells. They concluded that AQP 9 is markedly more highly expressed in the tumour progenitor population, whilst AQP4 is downregulated in tumour-derived differentiated cells. They proposed that AQP 9 may have a central role in the tumorigenesis of GBM [71].

7. Midkine

Midkine (MK) with the molecular weight of MK is 13 kDA is a heparin-binding growth factor/an angiogenic factor with cytokine actions. MK binds to oversulfated structures in heparan

sulfate and chondroitin sulfate. MK and pleiotrophin (known as PTN and HB-GAM) are belonging to same family [72]. MK is 50% homologous to PTN at the amino acid level and shares with PTN the genomic organization and predicted protein structure [72-73].

MK is mainly composed of two domains which are linked by disulfide bonds [74]. The C-domain has basic heparin-binding activity and this is responsible for the mechanism of action [75]. Each domain of MK has also homology to the thrombospondin Type I repeat [76]. Two domains are composed of three anti-parallel β-sheets [77]. The C-domain has two clusters of basic amino acids named as Cluster-1 and -2. These clusters are required for heparin-binding activity [78]. MK forms dimers via spontaneous association and transglutaminase stabilize dimers through crosslinking process. MK is seemed to require dimerization for its activity [79]. After dimerization, Cluster-2 forms a fused strong binding site [77].

MK was originally reported to be the product of a retinoic acid-responsive gene during embryogenesis [80]. its expression was high during embryogenesis, but interestingly, MK is not detectable in healthy adults and only re-appears in the body as a part of the pathogenesis of diseases [81]. MK promotes proliferation, migration, anti-apoptotic manner, mitogenesis, transforming, and angiogenesis various cells [82-87]. It has significant roles in reproduction, repair and in epidemiology of many diseases as rheumatoid arthritis, multiple sclerosis, hypertension and renal disease and cancer [88-90]. It's very important data that the expression of MK is increased in advanced tumors with high frequency [84, 91]. Previous reports showed that the blood MK level is frequently elevated with advance of human carcinomas, decreased after surgical removal of the tumors [91,92].

Human MK recognizes glycosaminoglycans through its C-domain as heparan sulfate trisulfated unit and chondroitin sulfate E unit is important in its mechanism of action. The component of the MK receptor is a chondroitin sulfate proteoglycan protein tyrosine phosphatase-z (PTPz). Low density lipoprotein receptor-related protein (LRP), α4β1-integrin and α6β1-integrin are also MK receptors [93,94].These proteins and PTPz form a receptor complex of MK. After the complex formation with PTPz and integrins, MK starts downstream signaling systems as Src family kinases and tyrosine phosphorylation,respectively. Increased tyrosine phosphorylation of paxillin leads to migration at osteoblast like cells and followed by suppression of caspases, activation of PI3 kinase and MAP kinase takes part in survival [83, 93, 95]. The previous report showed that when MK binds to a6b1-integrin and tetraspanin, and induces tyrosine phosphorylation of focal adhesion kinase (FAK) followed by activation of paxillin and signal transducer and activator of transcription (STAT) 1 alpha pathway, it increases migration and invasion at human head and neck squamous cell carcinoma cells in vitro [96]. Due to phosphorylation of STAT3 by MK, the proliferation of postconfluent 3T3-L1 cells are stimulated and this leads to adipogenesis [97]. Notch2 reserves an another receptor for MK and acting through the janus kinase 2 (Jak2)/STAT3 signalling pathway, MK leads to epithelial-mesenchymal transition (EMT) in immortalized keratinocytes. Both MK and PTN plays important role in EMT and neurogenesis during organogenesis process in embryonal development [96]. Previous reports proposed that anaplastic lymphoma kinase (ALK) can be included in the receptor group of MK [98]. Muramatsu and coworkers suggested that, ALK also involves in the MK complex with LRP and integrins that it is recruited to the receptor

complex and plays roles in MK signaling [99]. After activation by MK, ALK phosphorylates insulin receptor substrate-1, activates MAP kinase and PI3 kinase leading to transcriptional activation of Nuclear Factor-KappaB (NF-κB) [98].

MK binds to nucleolin, a nuclear protein which is also located at the cell surface and functions as a shuttle to the nucleus [85]. A component of the MK receptor LRP has major function as endocytose and delivering its ligands to lysosomes for degradation or catabolism [100]. LRP takes part in internalization of MK [101]. MK is not internalized in LRP-deficient cells, whereas transfection of a LRP expression vector can restore MK internalization and subsequent nuclear translocation, suggesting that LRP binds to MK and mediates nuclear targeting by MK. After this internalization, nucleolin transfer cytoplasmic MK to the nucleus [101]. With respect to nuclear targeting by MK, laminin-binding protein precursor (LBP) binds to MK and is cotranslocated with MK into nuclei [102]. MK may use both nucleolin and LBP precursor as shuttle proteins, revealing a novel role of LRP in intracellular signaling by its ligand, and the importance of nucleolin and LBP in the process of nuclear target of MK. MK transferred to the nucleolus is involved in the synthesis of ribosomal RNA [85]. Muramatsu and coworkers observed, however didn't publish that translation initiation factor (eIF3) can be an MK-binding protein in the embryonic brain [31].

8. MK and GBM

In the central nervous system, MK is expressed by astrocytes in the fetal brain and its expression is developmentally regulated, decreasing progressively to an undetectable level as the fetus matures [103,104]. Previous reports showed that increased levels of MK expression correlate with the progression of human astrocytomas, MK mRNA and protein expression levels were higher in high-grade astrocytomas as anaplastic astrocytomas and GBMs than in low-grade astrocytomas (oligodendroglioma, ependioma, schwannoma, meningioma and pituitary adenoma) [105]. These reports conclude that MK correlates with the poor prognosis of GBM.

One of the report showed that MK activates PI3-kinase and MAP kinase signal transduction in U87MG human glioblastoma cells which express ALK protein [98]. In this report it was shown that MK is also unable to stimulate Akt phosphorylation upon reduction of ALK. In their report they revealed that in contrast with the diminished PTN and MK signals after reduction of ALK, Akt phosphorylation in the same cells via a different tyrosine kinase receptor, the platelet-derived growth factor receptor (PDGF-R), was not altered by the reduction of ALK levels [107]. Interestingly, in the U87MG cells MAPK is activated constitutively and remains unaffected by the ALK reduction or by MK addition. In contrast to other report showed that no mRNA levels of ALK and RPTP β/ς levels, but high mRNA levels of MK and PTN were determined in another human GBM cell lines named T98G [98, 107]. This condition is also same for human glioblastoma cell lines named G55T2. U118 GBM cells possess high mRNA levels of ALK, low mRNA levels of MK and RPTP β/ς but no mRNA levels of PTN are detected. All cell lines derived from

human GBMs are different. Autophagy can both lead to cell death (autophagic cell death or apoptotic cell death) and cell survival (survival/recurrence/resistance). This means it becomes sometimes foe sometimes friend. Lorente and coworkers showed that activation of the tyrosine kinase receptor ALK by its ligand MK interferes with the signaling mechanism by which Δ9-tetrahydrocannabinol (THC) which is the main active component of marijuana, promotes cancer cell death via autophagy stimulation [108].

GBM has a complex tumor structure consisting of accumulating tumors cells, abnormal vessel and necrotic debris. The increasing tumor mass leads to increased capillary and venous collapse [109]. The new formed vessels are structurally and functionally abnormal, and leaky, leading to edema, and low oxygen tension [110]. High O2 tension degrades HIF-1α and consequently promotes differentiation or apoptosis, HIF-1α maintains at lower O_2 tension this augments signal transduction pathways leading to promote self-renewal [111]. Hypoxia induces MK expression through the binding of to a hypoxia responsive element in the MK promoter.

Notch2 has been suggested to lead embryonic brain tumor growth, however Notch3 has been implicated in choroid plexus tumors [112]. The frequency and the intensity of Notch2 expression is higher than that of Notch1 in GBM and in medulloblastoma [113]. As a consequence of local genomic amplifications at the Notch2 locus in both brain tumor types, this may also be linked to the later persistence of Notch2 expression in postnatal mouse brain [114]. Previous report showed that Notch1 regulates transcription of the epidermal growth factor receptor gene EGFR, known to be overexpressed or amplified in GBM, through TP53 [115]. Reports showed that there is a direct correlation between p53 and MK levels. Consistently, transcription of Notch signaling mediator genes are significantly overexpressed in the molecular subset of GBM with EGFR amplification [116]. Notch signaling pathway activates the major GBM signalling pathway. Glioma subsets with impaired Notch signaling have slower progression.

The most frequent genetic alteration occurring in GBM is genomic amplification of EGFR [117]. Consistently, EGF is the major proliferation pathway in GBM, mediated by activation of the RAS-RAF-MEK-ERK and the PI3K-AKT-mTOR cascades [58]. Interestingly, mTOR has recently been shown to activate Notch signaling in lung and kidney tumor cells through induction of the STAT3/p63/Jagged signaling cascade [118]. Lino and coworkers proposed this cross-talk for GBM that this suggests potential creation of a positive feedback loop between Notch and EGF signalling [119]. The most frequent GBM subset consists of the association of EGFR amplification, homozygous deletions at the cyclin dependent kinase 2A (CDKN2A) locus, and TP53 mutations [120]. Notch activates expression of EGFR via TP53 thus Notch is expected to stimulate the main GBM proliferation pathway [116]. In addition, Notch also transactivates the gene for the EGFR-related ERBB2 in a DTX1-dependent manner [121]. Notch-2 serves another receptor for MK and so cross-talk between MK and Notch-2 has been also shown to be a mediator of chemotherapy resistance to neighboring cells in GBM [122].

When a subset of cells overexpress drug transport proteins, possess receptor changes for the commitment of drug bounding and lack of ability to commit apoptosis, this situation leads to tumors resistance during chemotherapy. Mirkin and coworkers investigate the cytoprotective relationship between resistant and nonresistant cells in tumors which both accomplish to

survive against drug cytotoxicity in human neuroblastoma and osteosarcoma [123]. They hypothized that drug-resistant cells may secrete in their culture medium factors able to protect sensitive cells from cytotoxicity of drug. They showed that expression of MK was only detected in drug resistant cells and MK-enriched fractions exert a significant cytoprotective effect against doxorubicin (DXR) in the wild-type drug-sensitive cells. In addition, they transfected these cells with MK gene resulting in decreased response to DXR due to activation of AKT pathway and supression of caspase pathway. They concluded that the existence of intercellular cytoprotective signals such as the one mediated by MK, originating from cells with acquired drug resistance to protect neighboring drug-sensitive cells and thus contribute to development of resistance to chemotherapy. They didn't show anything aout direct effect of MK on drug efflux transporters.

Report by Hu and coworkers showed that the possible effects of MK gene on the chemotherapeutic drugs efflux. They concluded that there was powerful drug efflux ability in lymphoblastic leukemia cells with high MK gene expression [124]. They proposed that MK gene expression regulates drug efflux upstream of the p-gp and the other transporter proteins in this cell line. Previous reports showed that the expression of MRP-1 is higher than expression of p-gp in T98G [125]. In our study, we investigated whether the combination of an antineoplastic imatinib mesylate (IM) and an antitussive noscapine (Nos) with new identified chemotherapeutic effects, can be an effective GBM treatment and the role of MK in this treatment by using T98G cells [126]. The lowest MRP-1 levels, but highest MK levels were detected in the combination group. The lowest MK levels were detected in IM group especially at the 72nd hr ($p<0.05$) but IM takes second place at MRP-1 inhibition. The highest and the lowest p-170 levels were detected at IM group ($p<0.05$) and Nos group ($p<0.05$), respectively. Thus, we can conclude that drug efflux ability was not correlated with MK levels in this experiment.

Suppression of PTN and ALK expression has already been employed as means to treat GBM, and promising results have been obtained in animal experiments [107].

9. MK and GBM stem cells

Previous publication showed that MK is expressed in mouse embryonic stem cells (mESCs), human embryonic stem cells (hESCs) and mouse embryonic fibroblasts (MEFs) [127]. In their study, MK promotes proliferation and self-renewal of both mESCs and hESCs. Another study showed that the promoted growth of mESCs by MK is occured through inhibiting apoptosis while accelerating the progression toward the S phase, and MK leads to enhancement of mESC self-renewal through PI3K/Akt signaling pathway. They concluded that MK plays profound roles in ESCs and MK/PTPzeta signaling pathway is a novel pathway in the signal network maintaining pluripotency of ESCs. Their results gives more detailed information about the pluripotency control of ESCs and the relationship between ESCs and cancers. Huang and coworkers showed that a highly tumorigenic subpopulation of cancer cells named GBM stem cells (GSCs) promotes therapeutic resistance [127]. In their study, they showed that GSCs stimulate tumor angiogenesis by expressing elevated levels of VEGF and contribute to tumor

growth. In addition, cancer stem cells have been shown to promote metastasis. MK was found to be expressed in neural precursor cells, which consist of neural stem cells and the progenitor cells which has been translated into a useful therapeutic strategy in the treatment of recurrent or progressive GBMs [128].

10. MK and AQPs

Hypoxia is the intersection point for AQP and MK. Hypoxia increases all these protein levels as I mentioned above: their levels are all increased under hypoxic conditions. HIF-1α binds promoter of MK and AQP, then it increases its expression. In addition, HIF-1α serves as an upstream regulator of cerebral glycerol concentrations and brain edema via a molecular pathway involving AQP4 and AQP9 [70].

In our previous study published in Oncology Letter, we investigated the combination of imatinib (IM) and roscovitine (ROSC) to overcome resistance and whether or not MK had an effect on this combination in the treatment of GBM with other anti-apoptotic factors such as AQP4 in T98G human GBM cells. These cells are expressing high MK and AQP4 levels. In this study, all applications decreased the cell proliferation index and increased the apoptotic index, but ROSC was the most efficient drug and the second most efficient drug was IM to decrease cell proliferation and induce cell death. Combination therapy showed antagonist manner. Notably, ROSC increased AQP-4 levels, however it decreased MK levels. The combination group induced highest decrease in p170 levels (p<0.05), the second one was determined as the IM group (p<0.05). All drug applications decreased MRP-1 levels (p<0.05), but the highest decrease was determined in the combination group and the latter was IM (p<0.05). IM decreased AQP-4 levels, however the combination group and ROSC increased AQP4 levels in T98G GBM cells. This increase was higher in the combination group.

In our other study, we combined IM with lithium chloride (LiCl) in T98G cells [unpublished data]. This shows combination also showed antagonist effect. MRP-1 levels were decreased by LiCl, the combination group and IM, respectively. Firstly the combination group and secondly IM decreased p170 levels efficiently, but LiCl didn't make any change on these levels. Firstly LiCl and secondly the combination group induced highest decrease in AQP4 levels for 72 h. For MK levels, the decrease rate from highest to lowest were IM, LiCl and the combination group [unpublished data].

In this studies, you can see that we only searched for correlation between MK and AQP4 and there were no hypoxic conditions or no three dimensional cell culture model which hypoxic center is formed without hypoxic conditions. This means that we might have different results if 1) we used this model, 2) investigated other types of AQPs as AQP9, 3) use different GBM cell lines for these novel combinations.

Acknowledgement

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I declare that I have no competing interests.

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