NEOPLASIA
www.neoplasia.com

Volume 10 Number 3 March 2008 pp. 198–206 198

Review Article

The Sodium Pump α_1 Subunit as a Potential Target to Combat Apoptosis-Resistant Glioblastomas[1]

Florence Lefranc*,†,2 and Robert Kiss†,2

*Department of Neurosurgery, Erasme University Hospital, Université Libre de Bruxelles, Brussels, Belgium;
†Laboratory of Toxicology, Institute of Pharmacy, Université Libre de Bruxelles, Brussels, Belgium

Abstract

PURPOSE: To review the involvement of the ion transporter Na^+/K^+–ATPase (NaK) in the migration and proliferation of glioma cells. Preliminary studies indicate that NaK α_1 subunits seem to be upregulated in a proportion of glioblastomas but not in normal brain tissues. *DESIGN:* The present review focuses on (1) the natural resistance of migrating malignant glioma cells to apoptosis, (2) autophagic cell death as an alternative to combat malignant gliomas, (3) the fact that reducing the levels of malignant glioma cell motility can restore proapoptotic drug sensitivity, and (4) on the observation that inhibiting the NaK activity reduces both glioma cell proliferation and migration. *RESULTS:* The natural ligands of the NaK are the cardiotonic steroids. A hemisynthetic derivative of 2″-oxovoruscharin (UNBS1450), a novel cardenolide, displays unique structural features, making its binding affinity to NaK α subunits (including α_1) 10 to 100 times higher than that of other cardenolides. UNBS1450 markedly decreases intracellular ATP concentration in glioma cells, disorganizes the actin cytoskeleton, and leads to autophagic cell death in NaK α_1 over-expressing glioma cells. *CONCLUSIONS:* Glioblastoma patients who do not respond to chemotherapy and whose tumors over-express NaK α_1 subunits could benefit from a treatment using ligands with marked binding affinity for the NaK α_1 subunit.

Neoplasia (2008) 10, 198–206

Introduction

Gliomas account for more than 50% of all primary brain tumors and are by far the most common primary brain tumor in adults [1]. Despite the advances in the management of malignant gliomas, of which glioblastomas represent the ultimate grade of malignancy, they remain characterized by dismal prognoses [1–4]. Glioblastoma patients have a median survival expectancy of only 14 months on the current standard treatment of surgical resection to the extent feasible, followed by adjuvant radiotherapy plus temozolomide, given concomitantly with and after radiotherapy [1–3,5]. Malignant gliomas are associated with such dismal prognoses because glioma cells can actively migrate through the narrow extracellular spaces in the brain, often traveling relatively long distances, making them elusive targets for effective surgical management [1,2]. In addition, after surgical resection and adjuvant treatment of malignant gliomas, the residual cancer cells peripheral to the excised lesion give rise to a recurrent tumor, which, in more than 90% of cases, develops immediately adjacent to the resection margin [2,6,7].

Clinical and experimental data have also demonstrated that invasive malignant glioma cells show a decrease in their proliferation rates and a relative resistance to apoptosis compared to the highly cellular center of the tumor, and this may contribute to their resistance to conventional proapoptotic chemotherapy and radiotherapy [2,6,7]. As recently indicated by both Okada and Mak [8] and ourselves [2,9], despite this resistance to apoptosis being closely linked to tumorigenesis, tumor cells can still be induced to die by nonapoptotic mechanisms, such as necrosis, senescence, autophagy, and mitotic catastrophe. An international clinical trial [5] has recently revealed that the addition

Abbreviations: AMOG, adhesion molecule on glia; MGMT, methylguanine-DNA methyltransferase; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor–kappa B; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog on chromosome ten

Address all correspondence to: Florence Lefranc, MD, PhD, Department of Neurosurgery, Erasme Academic Hospital, Free University of Brussels, 808 route de Lennik, 1070 Brussels, Belgium. E-mail: fllefran@ulb.ac.be

[1]The present work was supported in part by grants awarded by the Fonds Yvonne Boël (Brussels, Belgium) and by the Région de Bruxelles-Capitale (Brussels, Belgium).
[2]R.K. is a Director for Research and F.L. is a Clinical Research Fellow with the Fonds National de la Recherche Scientifique, Belgium.
Received 22 October 2007; Revised 17 December 2007; Accepted 18 December 2007

DOI 10.1593/neo.07928

of the chemotherapeutic agent temozolomide to radiation therapy increases survival of patients suffering from glioblastoma. A companion laboratory study [10] has offered hope of even greater improvements in patient survival in the future, through the identification of a molecular change in the tumor that will permit the prediction of the benefit of this new combined treatment. Temozolomide may thus circumvent part of the glioblastoma resistance to apoptosis [2,11].

Another potential means of overcoming apoptosis resistance is by decreasing the migration of migrating glioma cells, which results in a significant increase in the level of sensitivity of these cells to proapoptotic drugs [2,6]. Glioma cells are *self-propelled* [12] and are able to adjust their shape and volume rapidly as they invade the brain parenchyma. Essential to this process is the activity of chloride channels, anion transport mechanisms [13], and aquaporins [14]. The sodium pump is another ion transporter that, in addition to exchanging cations, is also directly involved in the migration of cancer cells in general [15–17] and of glioma cells in particular [18–20]. The present review emphasizes the fact that a cardenolide-mediated decrease in sodium pump activity could be used to combat apoptosis-resistant malignant gliomas.

Natural Resistance of Migrating Malignant Glioma Cells to Apoptosis (Radiotherapy and Chemotherapy)

The natural resistance of glioblastomas to radiotherapy and chemotherapy is attributed, at least partly, to the phosphatase and tensin homolog on chromosome ten (PTEN)/Akt/phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR)/nuclear factor–kappa B (NF-κB) pathway [2,9,21–26] (Figure 1). The activity of the PI3K/Akt pathway is often upregulated in brain tumors due to excessive stimulation of growth factor receptors and Ras [27,28]. The *PTEN* tumor suppressor gene mutations, which result in the activation of the PI3K-dependent activation of Akt signaling [27,29], are frequent in *de novo* glioblastomas [29]. Methylation of the *PTEN* promoter may represent an alternate mechanism by which PI3K signaling is increased in grade II and III gliomas as well as in secondary glioblastomas [24]. The activation of the PI3K pathway is associated significantly with increasing tumor grade, lower levels of apoptosis, and an adverse clinical outcome in the case of human gliomas [30]. Narita et al. [31] and Choe et al. [32] suggest that the PI3K/Akt pathway is a particularly interesting target in the case of glioblastomas with aberrant epithelial growth factor receptor (EGFR) expression, because the aberrant EGFR expression and abnormal PI3K/Akt signaling also modulate the levels of migration of tumor astrocytes [2]. A number of publications have already reported that an aberrantly activated PI3K/Akt pathway renders tumor cells resistant to cytotoxic insults, notably to anticancer drugs [33,34]. Shingu et al. [34,35] have shown that the inhibition of this pathway restores or even augments the effectiveness of chemotherapy on glioma cells. PI3K inhibitors could also be used to reduce the levels of tumor astrocyte migration, a feature that could restore a certain level of apoptosis to these cells [33]. Cell survival through Akt signaling also involves the NF-κB pathway because Akt signals to various cell death regulators including IκB kinase, which controls NF-κB activity. NF-κB itself plays a dramatic role in gliomagenesis [2,23]. For example, the NF-κB signaling pathway is constitutively activated in a large proportion of glioblastomas [36], and this activation enables cancer cells to resist cytotoxic insults [37,38]. The constitutive activation of Akt and NF-κB contributes significantly to the progression of diffuse gliomas, and the activation of Akt may lead to NF-κB activation in high-grade gliomas. As detailed below, cardenolide-induced inhibition of Na^+/K^+–ATPase (NaK) activity leads to the deactivation of the NF-κB pathway.

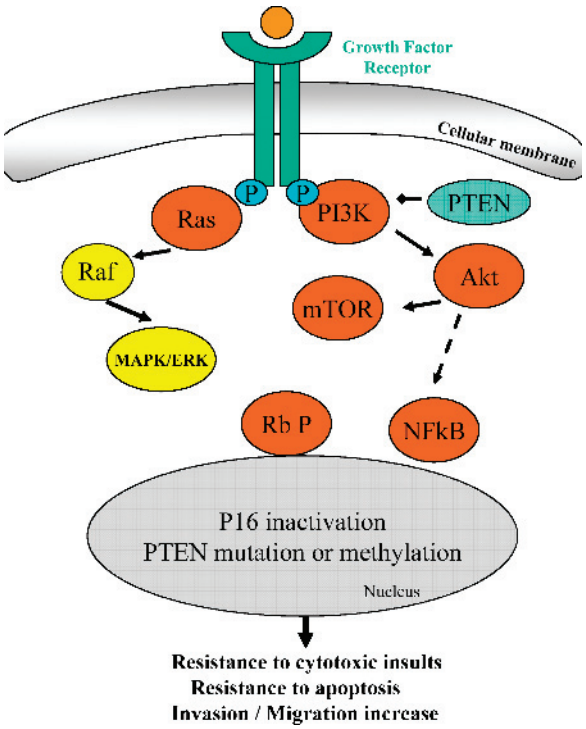

Figure 1. Pathways involved in the natural resistance of glioblastomas to radio/chemotherapy. Pathways involved in cytotoxic insult resistance are presented in orange. The *PTEN* gene mutation or methylation promotes Akt activation. ERK indicates extracellular signal–regulated kinase; MAPK, mitogen-activated protein kinase.

Rapamycin inhibits the phosphorylation of the retinoblastoma protein, and rapamycin-treated glioblastoma cells are therefore not fully committed to entering the S-phase after their release from drug-induced G_1 arrest [39,40]. Constitutive Rb phosphorylation frequently occurs in glioblastomas due to mutation-induced *p16* gene inactivation [1]. The mTOR can also control cell migration in glioblastomas [2].

Several strategies could be used potentially to overcome glioma cell resistance to cytotoxic insults: (1) inhibition of the signaling pathways that are constitutively activated in a specific malignant glioma specimen; (2) use of inhibitors to reduce the levels of tumor astrocyte migration, a feature that could restore a certain level of apoptosis to these cells; (3) induction of autophagic cell death as opposed to apoptosis; and (4) inhibition of sodium pump activity, which disorganizes the actin cytoskeleton (reducing motility and proliferation) and induces autophagic processes in malignant glioma models [2].

Autophagy: A Potential Trojan Horse for Malignant Gliomas

Cell death can be divided into apoptotic cell death and nonapoptotic cell death [8]. Because glioblastoma cells carry mutations that inactivate apoptotic pathways [2], nonapoptotic cell death could represent an alternative for apoptosis-resistant glioma cells to be destroyed [9,41]. Whereas apoptosis is a caspase-dependent process characterized by the condensation of cytoplasm and the preservation of organelles, essentially without any autophagic degradation, autophagic cell death

is a caspase-independent process that exhibits extensive autophagic degradation of the Golgi apparatus, the polyribosomes, and the endoplasmic reticulum (Figure 2), with all these features preceding the destruction of the nucleus [8,9,42]. Autophagy begins with the sequestration of cytoplasmic organelles into membrane vacuoles called autophagosomes which then fuse with lysosomes to form autolysosomes, in which materials are subsequently degraded and recycled [9,11,42] (Figure 2). The mTOR seems to be involved in autophagic cell death as its inactivation can induce autophagy [9] (Figure 2). The mTOR is a downstream effector of the PI3K/Akt signaling pathway, and it is also a central modulator of cell proliferation in malignant gliomas [40,43]. In fact, rapid tumor proliferation (that can result from low apoptotic levels) may contribute to the clinical resistance of glioblastomas to radiotherapy, and disruption of mTOR signaling by rapamycin appears to return a certain level of sensitivity to resistant glioblastoma cells [44]. Takeuchi et al. [43] showed that rapamycin induced autophagy but not apoptosis in rapamycin-sensitive malignant glioma U87-MG and T98G cells by inhibiting the function of mTOR. In contrast, in rapamycin-resistant U373-MG cells, the inhibitory effect of rapamycin is minor [43].

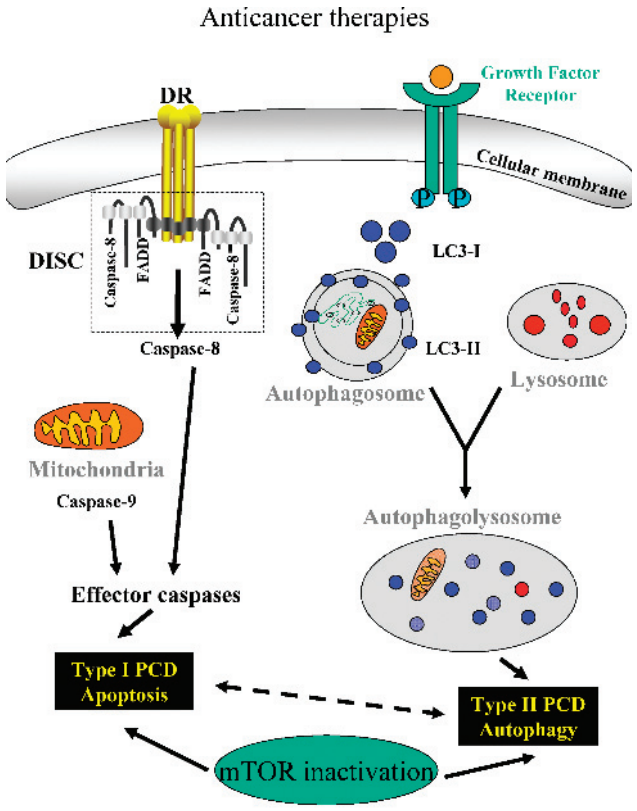

Figure 2. The molecular regulation of autophagy and the link with apoptosis. In the presence of growth factors, growth factor receptor signaling activates cascades to the mTOR, resulting in the inhibition of autophagy or type II programmed cell death (PCD; right-hand side of the figure). In contrast, inactivation of mTOR can induce both apoptosis and autophagy. The left-hand side of the figure represents a simplified version of the two main signaling pathways of apoptosis. The intrinsic pathway includes the mitochondria. The extrinsic cell death pathway is mediated by death receptors (DR). After formation of the DR/death-inducing signaling complex (DISC) and recruitment of caspases, downstream signaling pathways lead to apoptosis.

A source of real hope in glioma chemotherapy is offered by temozolomide, a second-generation imidazotetrazine-alkylating agent [3,5,45]. Temozolomide is a small lipophilic molecule, which can be administered orally and which crosses the blood-brain barrier effectively. Moreover, temozolomide is less toxic to the hematopoietic progenitor cells than conventional chemotherapeutic agents and does not require any hepatic metabolism for activation [11]. An international clinical trial [5] has shown that the addition of temozolomide to radiotherapy increases the survival of patients suffering from newly diagnosed glioblastomas. Indeed, addition of temozolomide to radiotherapy improves median survival in newly diagnosed glioblastoma patients from 12.1 to 14.6 months, and it increases the 2-year survival from 10.4% to 26.5% [5]. Part of temozolomide's cytotoxic activity is exerted through proautophagic processes, at least in glioblastoma cells, due to the formation of O^6-methylguanine in DNA, which mispairs with thymine during the next cycle of DNA replication [11,46]. Glioma cells thus respond to temozolomide by undergoing G_2/M arrest but will ultimately die from autophagy [11,46]. Knowing that O^6-alkylguanine-DNA alkyltransferase is a DNA repair enzyme that limits the efficacy of temozolomide in glioblastoma cells, Kanzawa et al. [46] showed that inhibition of O^6-alkylguanine-DNA alkyltransferase by O^6-benzylguanine can render previously resistant glioblastoma cells sensitive to temozolomide. Hegi et al. [10] showed that patients who had glioblastomas that contained a methylated O^6-methylguanine-DNA methyltransferase (MGMT) promoter benefited from temozolomide, whereas those who did not were less responsive. However, results contradicting these findings are present in the literature, and the clinical and genetic context framing MGMT methylation is poorly characterized. Recent observations suggest that MGMT methylation is part of the genetic signature of glioblastomas that develop from lower-grade gliomas [47]. Part of temozolomide's cytotoxic activity is, however, also due to the induction of late apoptosis. Indeed, Roos et al. [48] showed that malignant glioma cells undergo apoptosis after treatment with the methylating agents *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and temozolomide. O^6-methylguanine–triggered apoptosis in gliomas is a late response (occurring >120 hours after treatment) that requires extensive cell proliferation. Overall, the data reported by Roos et al. [48] demonstrate that cell death induced by temozolomide in gliomas is due to apoptosis and that the determinants of sensitivity of gliomas to temozolomide are MGMT, p53, proliferation rate, and double-strand break repair.

The data reported by Kanzawa et al. [11] and Roos et al. [48] are not contradictory, because autophagy and apoptosis may be triggered by common upstream signals, and sometimes this results in combined autophagy and apoptosis [49] (Figure 2). In other instances, the cell switches between the two responses in a mutually exclusive manner [49]. On a molecular level, this means that the apoptotic and autophagic response machineries share common pathways that either link or polarize the cellular responses [49] (Figure 2). We will show below that inhibiting sodium pump activity in human apoptosis–resistant glioblastoma cells can induce marked processes of autophagy.

Reducing Malignant Glioma Cell Motility to Restore Proapoptotic Drug Sensitivity

For motility, cells must acquire spatial asymmetry enabling them to turn intracellularly generated forces into net cell body translocation [2]. One characteristic of this asymmetry is a polarized morphology, i.e., a clear distinction between the front and rear of cells. An

early event in this polarization is a change in filamentous F-actin distribution from azimuthal symmetry around the cell rim to a concentration in a particular region [2]. Additional molecular rearrangements consist of the redistribution of chemosensory signaling receptors, integrins, and other adhesion receptors, and the redistribution of integrin-cytoskeleton linkages [2]. Another important consequence of polarization is that the extension of active membrane processes, including both lamellipodia and filopodia, takes place primarily around the cell front, so that directional turning is generally accomplished gradually, with cell locomotion taking on the character of a persistent random walk. Lamellipodia are broad flat sheet-like structures, whereas filopodia are thin cylindrical needle-like projections. These structures contain an abundance of actin and actin-associated proteins [2]. The extension of both the lamellipodia and the filopodia in response to migratory stimuli is almost universally found in conjunction with local actin polymerization [2,50,51]. Along with a bias toward membrane extensions at the cell front, attachments tend to form preferentially at the leading edge of lamellipodia and filopodia [2,50,51]. Rapid migration also requires efficient mechanisms to release adhesion at the rear [2]. Whereas the actin cytoskeleton provides the driving force at the front, the microtubule network assumes a regulatory function in coordinating rear retraction [2].

As indicated previously, one potential way of overcoming apoptosis resistance is by decreasing the migration of migrating glioma cells, which results in a net increase in the level of sensitivity of these cells to proapoptotic drugs [2]. This concept was recently verified *in vivo* after combining temozolomide with two distinct antimigratory strategies. Indeed, we showed that when compared to temozolomide alone, the therapeutic benefits obtained *in vivo* in experimental models of human orthotopic glioblastomas were significantly more pronounced when combining either cimetidine (a histamine H2 receptor antagonist with antiadhesive properties) [52] and temozolomide [53] or temozolomide and a small interfering RNA–directed against galectin-1 [54], which is a potent modulator of glioma cell migration [55]. Similar data were obtained when we combined temozolomide with the targeting of sigma1 receptors in glioblastoma cells [56].

The Sodium Pump Constitutes a New Target to Combat Malignant Gliomas

The Sodium Pump

In mammalian cells, active sodium transport and its derived functions (e.g., plasma membrane potential) are dictated by the activity of the NaK, whose regulation is essential for maintaining cell volume and composition, as well as other vital cell functions [57]. Because the plasma membrane is highly permeable to water, it is the concentration of ions across this membrane that is, in the short term, critical for maintaining an adequate cell volume. The plasma membrane NaK is important in this process because it provides the driving force for active sodium and potassium transport into and out of the cell, with water following isosmotically [57]. Increases in sodium permeability require concomitant increments in NaK-mediated outward sodium transport to prevent a disproportionate increase in the intracellular sodium concentration/osmotic pressure and, consequently, cell swelling (Figure 3*A*).

The sodium pump consists of equimolar ratios of two main subunits, the catalytic α and regulatory β polypeptides, each of which exists as several isoforms [17]. To date, four different α and three distinct β isoforms have been identified in mammalian cells [17,58–60]. The α subunit contains the binding sites for the previously mentioned cations, ATP, and cardiotonic steroid inhibitors [18,59,60]. Whereas the β subunit is essential for the normal activity of the enzyme, it also appears to be involved in the occlusion of K^+ and modulation of the enzyme's K^+ and Na^+ affinity [18,60]. In addition to pumping ions (Na^+ and K^+) across the plasma membrane (Figure 3*A*), the sodium pump functions as a receptor for cardiotonic steroids [17,59,60], including ouabain, digitoxin, and digoxin, and the novel cardenolide UNBS1450 [17,18,20] (Figure 3).

During apoptosis, there is compelling evidence indicating an early increase in intracellular sodium followed by a decrease in both intracellular K^+ and Na^+, suggesting a regulatory role for these cations during both the initial signaling and the execution phase of apoptosis [60]. Recent studies have shown that the NaK is involved in controlling perturbations of Na^+ and K^+ homeostasis during apoptosis and that antiapoptotic Bcl-2 and Bcl-X_L molecules influence these ionic fluxes.

Cardiotonic Steroids: Ligands of the Sodium Pump

Cardenolides and bufadienolides are the two chemical subclasses that constitute the cardiotonic steroids [17]. The sodium pump is characteristically inhibited by cardiac glycosides. By binding to NaK, cardiotonic steroids elicit the activation of the so-called *Na^+/K^+–ATPase signalosome* [17,58] (Figure 3*B*).

The growth of a glioma requires the destruction of the normal brain parenchyma by the glioma cells [12]. This is achieved through the cellular release of glutamate into the peritumoral space [61] in the absence of functional Na^+-dependent glutamate transporters in glioma cells, resulting in the consequent accumulation of excitotoxic glutamate in the extracellular glia space [62]. Signaling through glutamate receptors is also involved in glioblastoma cell proliferation [63]. In addition, as indicated in the Introduction, glioma cells are *self-propelled* [12] and are able to adjust their shape and volume rapidly as they invade the brain parenchyma. Essential to this process is the activity of Cl^- channels and anion transport mechanisms [13]. As indicated previously, NaK is an ion transporter, which, in addition to exchanging cations, is also directly involved in the migration of cancer cells in general [17,18] and of glioma cells in particular [20], as detailed in the next section. Moreover, the activity of NaK can be modulated by glutamate and its receptors [64].

Cardenolides, such as digitalis (digitoxin and digoxin), have been used for the treatment of congestive heart failure for more than 200 years [18]. Digoxin is still used to treat approximately 1.7 million patients in the United States for heart failure and/or atrial fibrillation despite the development of newer pharmacological agents, such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists, and β-blockers [18]. Besides its classic use in cardiac diseases, the use of digitalis in oncology has already been proposed [65–67]. As mentioned previously, the constitutive activation of the NF-κB pathway in cancer cells leads them to become chemoresistant [37,38]. Different types of cardenolides are able to suppress the constitutive activation of NF-κB [17,66,68] to induce apoptosis [17,67] and to overcome multidrug resistance [69]. Several cardenolides have been shown to display *in vitro* antitumor activities against various types of cancer cells [17,65–68] including glioma cells [20,69]. However, none have ever reached clinical application because either their therapeutic index (ouabain, digitoxin) or their antitumoral activity (digoxin) were too low. UNBS1450 is a novel cardenolide hemisynthesized from 2″-oxovoruscharin [69], with a

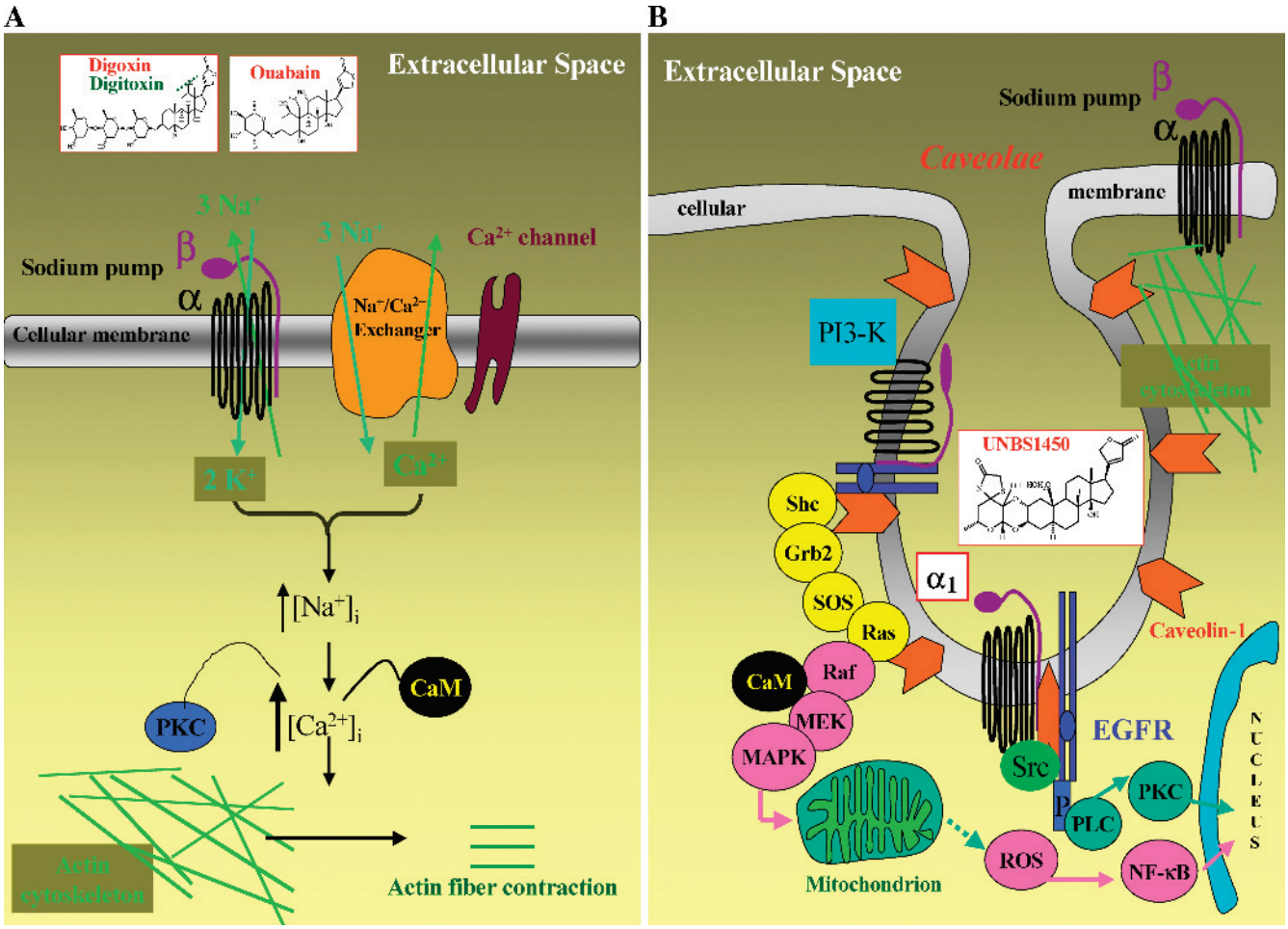

Figure 3. The NaK functions as a receptor for cardiotonic steroid inhibitors. In addition to pumping ions (Na^+ and K^+) across the plasma membrane using ATP as the driving force (A), the sodium pump in caveolae is engaged in the assembly of multiple protein complexes that transmit signals to different intracellular compartments (B). (A) For every three Na^+ ions pumped out of the cell, two K^+ ions are pumped in. The partial inhibition of the sodium pump by the cardenolide ouabain causes a modest change in $[Na^+]_i$ and $[K^+]_i$, and a significant change in $[Ca^{2+}]_i$ through the Na^+/Ca^{2+}–exchanger. (B) The NaK signalosome closely interacts with major components of gliomagenesis: EGFR, caveolin-1, PI3K, Src, and Ras. On ouabain binding, the NaK α_1 subunit in caveolae associates with several proteins, such as caveolin-1 through two caveolin-binding motifs and Src through multiple domains. Activated Src secondarily trans-activates EGFR which in turn recruits the adapter protein Shc to relay signals to the Ras-Raf-MAPKs cascade. In contrast, UNBS1450 at 10 nM, a concentration at which it demonstrates potent antiproliferative and antimigratory activity, does not elicit increases in $[Ca^{2+}]_i$ or $[Na^+]_i$ in cells but the compound decreases ($[ATP]_i$). Grb2 indicates growth factor receptor–bound protein 2; MEK, MAPK-ERK–activating kinase; PKC, protein kinase C; PLC, phospholipase C; Ros, reactive oxygen species; Shc, src homology collagen–like protein; Sos, son of sevenless.

toxicity profile similar to digoxin but with significantly more marked antitumor activity [17,70]. It displays potent antitumor activity in human non–small cell lung cancers [68,71], especially in those cancers in which the NaK α_1 subunit is over-expressed [17]. UNBS1450 induces nonapoptotic cell death in apoptosis-resistant cancer cells, including lysosomal membrane permeabilization–related death in non–small cell lung cancer cells [71] and autophagic cell death in glioblastoma cells [20].

The Sodium Pump Is Involved in Cancer Cell Proliferation, Migration, and Death

There are in fact two pools of the sodium pump within the plasma membrane with two distinct functions. One constitutes the energy-transducing pool of the enzyme broadly distributed in the plasma membrane (Figure 3*A*). The other is the signal-transducing pool of the enzyme restricted to the caveolae which is independent of changes in intracellular Na^+ and K^+ concentrations [58,60] and requires the initial association of the sodium pump with tyrosine kinase Src [72] (Figure 3*B*). The NaK signalosome closely interacts with major components of gliomagenesis, including for example, the interaction of EGFR with caveolin-1 [60], the binding of PI3K to NaK [16], and the involvement of Src, Ras, and EGFR in the signal-transducing function of NaK [72] (Figure 3*B*).

In carcinomas of epithelial origin, downregulation of β_1 subunit expression has been shown to induce an increase in the expression of the transcription factor Snail, which is known to downregulate E-cadherin [15] and so facilitate the spread of cancer cells from the primary tumor [16]. The β_2 isoform of the sodium pump is in fact a homolog of the adhesion molecule on glia (AMOG) which is a recognition element for cell adhesion that subsequently links cell adhesion and ion transport [19,73,74]. The AMOG/β_2 and the α_1 subunits of the sodium pump come together to form functional

sodium pumps [74]. The AMOG is downregulated in human and mouse gliomas [19]. We have identified the sodium pump as a major element in the migration of tumor cells [17,18], including glioblastoma cells [20] arising from its close interaction with caveolin-1 and its role in the organization of the actin cytoskeleton in the *caveolae*. We have found that the α_1 subunit of the sodium pump is located at the lamellipodia of the human glioblastoma cell line U373-MG, where it colocalizes with caveolin-1 [20]. Caveolae functions rely on caveolin-1, their major protein, which drives the formation of plasma membrane caveolae and anchors them to the actin cytoskeleton (Figure 3*B*). In addition, caveolin-1 modulates cell interaction with the extracellular matrix and brings together and regulates the interaction of different signaling molecules, with significant roles in cell movement [75]. Importantly, caveolin-1 depletion results in the loss of focal adhesion sites and overall cell adhesion [75]. Furthermore, we have shown by means of quantitative reverse transcription–polymerase chain reaction that the levels of α_1 subunit mRNA are higher in glioblastomas (7 of 10 human glioblastomas analyzed) than in normal brain tissue (0 of 4 normal brain tissue samples) [20]. Such measurements also revealed high levels of the α_1 subunit mRNA in human glioblastoma U373-MG cells [20].

UNBS1450: A Novel Cardiotonic Steroid That Potently Inhibits NaK α_1 Subunit Activity

We have shown that various types of cancer including glioblastomas [20], non–small cell lung cancers [17] and melanomas (manuscript in preparation) over-express the α_1 subunit of the sodium pump when compared to the normal tissues from which they arise. However, classic cardenolides display too poor an affinity for this subunit to make them potential antitumor agents acting through the inhibition of NaK α_1. However, UNBS1450, because of its marked inhibitory effects on NaK α_1 subunit activity [17,20], may represent such a potential candidate. To determine the effect of the compound on the activity of NaK isozymes, $\alpha_1\beta_1$, $\alpha_2\beta_1$, and $\alpha_3\beta_1$, sodium pump complexes were produced using a baculovirus expression system and dose-response curves to the compound were performed [17,20]. Heterologous protein expression in insect cells was used because it offered the advantage of a eukaryotic expression system able to produce large amounts of active recombinant NaK isozymes in a background with very little or no endogenous sodium pump present [58]. UNBS1450 had a marked inhibitory effect on $\alpha_1\beta_1$, $\alpha_2\beta_1$, and $\alpha_3\beta_1$ NaK isozymes [17,20]. The calculated inhibition constants (K_i) show that the compound inhibits $\alpha_1\beta_1$ with a potency that is approximately 100 times greater than that of ouabain or digoxin [17,20]. Taken together, these data suggest that the α_1 subunit of the sodium pump may indeed represent a potential target to combat apoptosis-resistant proliferating, as well as, migrating glioblastoma cells.

UNBS1450 Displays Marked Antiproliferative Effects In Vitro

UNBS1450 has been tested in 58 distinct human cancer cell lines and it displays antitumor effects similar to taxol [69]. It is also active on taxol-resistant tumor cells [69]. In three distinct glioblastoma cell lines, the *in vitro* antiproliferative effects of UNBS1450 are similar to vincristin but much greater than those displayed by temozolomide, tamoxifen, hydroxy-tamoxifen, lomustin, procarbazin, and carmustin [20]. All of these named drugs have been evaluated with varying degrees of success in the treatment of patients with malignant gliomas [2,3,76,77].

UNBS1450 is more potent in inhibiting the proliferation of U373-MG glioblastoma cells than that of normal cells [20], a feature that can be explained, at least in part, by the fact that (1) U373-MG glioblastoma cells express higher levels of NaK α_1 subunits than normal cells, (2) UNBS1450 decreases the intracellular ATP concentration ($[ATP]_i$) more markedly in U373-MG glioblastoma cells than in normal cells, and (3) $[ATP]_i$ is higher in normal cells than in U373-MG cancer cells [20].

UNBS1450 Contributes Significant Therapeutic Benefits in a Human Orthotopic Glioblastoma Xenograft Diffusely Invading the Brain Parenchyma of Immunocompromised Mice

Active doses of UNBS1450 in human cancer xenograft models *in vivo* are in the 10-to-20-mg/kg range on repeat intraperitoneal (i.p.) administration [68,70,71], whereas the acute lethal i.p. dose is ~120 mg/kg in mice [69]. We have also demonstrated that the compound is active at lower repeat-dose levels after IV dose against orthotopic human U373-MG glioblastoma xenografts [20]. The dose used was made of the U373-MG glioblastoma model because it displays astrocytic differentiation features [78,79], it is nondeleted in 1p19q [78], and it behaves as a chemoresistant high-grade malignant glioma [78] in which surgery is only of limited therapeutic benefit [80] given its diffusely invasive nature into the brains of immunocompromised mice [20]. In this U373-MG orthotopic xenograft model, UNBS1450 contributes therapeutic benefits that are similar or even higher than those of temozolomide [20].

Mechanism of Action of UNBS1450 in Glioblastoma Cells

Our results show that UNBS1450-mediated antiproliferative and antimigratory effects on human glioblastoma cells occur as a result of the disorganization of the actin cytoskeleton [20]. It should be borne in mind that the actin cytoskeleton is involved in many cellular processes that are essential for cell growth, differentiation, division, membrane organization, and motility [50]. Moreover, the association of actin filaments with the plasma membrane provides mechanical stability, maintains cell shape and adhesion, and regulates dynamic surface protrusions such as lamellipodia and filopodia, which are fundamental determinants of motility and migratory potential of cells [51].

As stated previously, the sodium pump is directly linked to the actin membrane cytoskeleton through its α subunit, and UNBS1450 markedly inhibits sodium pump activity (Figure 3) [69,70,81]. Furthermore, cellular ATP depletion results in a rapid duration-dependent dissociation of the sodium pump from the actin cytoskeleton [81] and in the redistribution of F-actin from a primarily cortical concentration to a perinuclear location [81], as observed when treating U373-MG glioblastoma cells with UNBS1450 [20]. The potent antiproliferative effects observed with UNBS1450 in three human glioblastoma cell lines were not related to proapoptotic effects at concentrations ranging between 10 and 1000 nM [20]. UNBS1450-induced cell death in these human glioblastoma cell lines seemed to occur as the result of proautophagic effects [20]. Indeed, we first observed an increase in acidic vesicular organelles (including autophagic vacuoles) in human glioblastoma cells treated with UNBS1450. The levels of microtubule-associated protein 1 light chain 3 and beclin-1, two specific autophagy markers [9,11] (Figure 2), also increased in human glioblastoma cells treated with UNBS1450 [20]. UNBS1450-induced decreases in $[ATP]_i$ could be also responsible, at least in part, for the induction of the autophagic processes [42,82]. Indeed, intracellular ATP depletion triggers autophagy through the mTOR pathway [42].

Novel Cardenolide Preclinical Data

Whereas UNBS1450 displays cardiovascular side effects that are similar *in vivo* in dogs to digoxin, it is between 20 and 100 times more potent against cancer cell lines *in vitro* [17]. The IC_{50} values for the antiproliferative activity of digitoxin *in vitro* range between 3 and 33 nM depending on the cancer cell line used, whereas peak concentrations in the plasma of patients with cardiac disease range between 20 and 33 nM [83]. The antiproliferative IC_{50} values of UNBS1450 range between 5 and 20 nM, however it is 10 times less toxic than digitoxin to mice [68]. Moreover, the plasma half-life of UNBS1450 is ~1 to 2 hours compared to about 8 hours for digoxin. UNBS1450 may therefore not present the potential inconvenience of cumulative cardiotoxicity seen with digoxin. The limiting toxicity of cardenolides is indeed their cardiotoxicity, which relates to the increase in $[Ca^{2+}]_i$ due to the cardenolide-mediated inhibition of the sodium pump. The increase in $[Ca^{2+}]_i$ leads to an increase in $[Na^+]_i$ and a decrease in the Na^+ gradient that drives the function of the Na^+/Ca^{2+} exchanger [18]. A lower activity of the Na^+/Ca^{2+} exchanger results in a rise in $[Ca^{2+}]_i$ and the positive inotropic effects of cardiotonic steroid [18]. It is this increase in $[Ca^{2+}]_i$ that is also involved in most of the adverse effects of digitalis. In contrast, UNBS1450 does not elicit increases in $[Ca^{2+}]_i$ or $[Na^+]_i$ in cells at its antiproliferative and antimigratory IC_{50} values [17,20].

UNBS1450 is currently undergoing regulatory preclinical evaluation (toxicology, safety pharmacology, drug metabolism, and pharmacokinetic development studies are ongoing) and should reach Phase I clinical trials by mid 2008.

Conclusions

Malignant gliomas are associated with dismal prognoses because they diffusely infiltrate the brain parenchyma, therefore rendering total surgical resection of all tumor cells impossible. In addition, those migrating glioblastoma cells that escape the most sophisticated surgical approaches are resistant to apoptosis and thus to radiotherapy and most chemotherapeutic agents. However, a number of strategies are emerging to overcome, at least partly, the resistance of migrating glioma cells to apoptosis. These involve (1) inhibition of the molecular pathways involved in apoptosis resistance that are overexpressed in glioma cells, (2) induction of autophagy, and (3) reducing malignant glioma cell migration; a process which in turn seems to restore a certain level of sensitivity to proapoptotic cytotoxics in migration-restricted glioma cells.

Our strategy has been to target the α_1 subunit of the sodium pump in those glioblastomas that over-express this subunit with novel, potent, and selective cardenolides. In so doing, using UNBS1450, it has been possible to markedly impair (at least in experimental models of human glioblastomas) both glioblastoma cell proliferation and migration (through a disorganization of the actin cytoskeleton), with marked features of autophagy as the terminal outcome. In essence, the targeting of the sodium pump α_1 subunit in glioblastoma cells appears to impair both their proliferation and migration, even if they are resistant to apoptosis.

References

[1] Kleihues P and Cavenee WK (2000). *Pathology and Genetics of Tumours of the Nervous System. International Agency for Research on Cancer (IARC) and WHO Health Organisation.* Oxford, UK: Oxford Press.

[2] Lefranc F, Brotchi J, and Kiss R (2005). Possible future issues in the treatment of glioblastomas: special emphasis on cell migration and the resistance of migrating glioblastoma cells to apoptosis. *J Clin Oncol* **23**, 2411–2422.

[3] Lefranc F, Sadeghi N, Camby I, Metens T, Dewitte O, and Kiss R (2006). Present and potential future issues in glioblastoma treatment. *Expert Rev Anticancer Ther* **6**, 719–732.

[4] Berens ME and Giese A (1999). "…those left behind." Biology and oncology of invasive glioma cells. *Neoplasia* **1**, 208–219.

[5] Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, et al. (2005).European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* **352**, 987–996.

[6] Giese A, Bjerkvig R, Berens ME, and Westphal M (2003). Cost of migration: invasion of malignant gliomas and implications for treatment. *J Clin Oncol* **21**, 1624–1636.

[7] Hoelzinger DB, Mariani L, Weis J, Woyke T, Berens TJ, McDonough WS, Sloan A, Coons SW, and Berens ME (2005). Gene expression profile of glioblastoma multiforme invasive phenotype points to new therapeutic targets. *Neoplasia* **7**, 7–16.

[8] Okada H and Mak TW (2004). Pathways of apoptotic and non-apoptotic death in tumour cells. *Nat Rev Cancer* **4**, 592–603.

[9] Lefranc F and Kiss R (2006). Autophagy, the Trojan horse to combat glioblastomas. *Neurosurg Focus* **20**, E7.

[10] Hegi ME, Diserens AC, Godard S, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, et al. (2004). Clinical trial substantiates the predictive value of *O*-6-methylguanine-DNA methyltransferase promoter methylation in glioblastoma patients treated with temozolomide. *Clin Cancer Res* **10**, 1871–1874.

[11] Kanzawa T, Germano IM, Komata T, Ito H, Kondo Y, and Kondo S (2004). Role of autophagy in temozolomide-induced cytotoxicity for malignant glioma cells. *Cell Death Differ* **11**, 448–457.

[12] Sontheimer H (2003). Malignant gliomas: perverting glutamate and ion homeostasis for selective advantage. *Trends Neurosci* **26**, 543–549.

[13] Ransom CB, O'Neal JT, and Sontheimer H (2001). Volume-activated chloride currents contribute to the resting conductance and invasive migration of human glioma cells. *J Neurosci* **21**, 7674–7683.

[14] Hayashi Y, Edwards NA, Proescholdt MA, Oldfield EH, and Merrill MJ (2007). Regulation and function of aquaporin-1 in glioma cells. *Neoplasia* **9**, 777–7878.

[15] Espinada CE, Chang JH, Twis J, Rajasekaran SA, and Rajasekaran AK (2004). Repression of Na,K–ATPase β_1-subunit by the transcription factor Snail in carcinoma. *Mol Biol Cell* **15**, 1364–1373.

[16] Barwe SP, Anilkumar G, Moon SY, Zheng Y, Whitelegge JP, Rajasekaran SA, and Rajasekaran AK (2005). Novel role for Na,K–ATPase in phosphatidylinositol 3-kinase signaling and suppression of cell motility. *Mol Biol Cell* **6**, 1082–1094.

[17] Mijatovic T, Roland I, Van Quaquebeke E, Nilsson B, Mathieu A, Van Vynckt F, Darro F, Blanco G, Facchini V, and Kiss R (2007). The alpha-1 subunit of the sodium pump could represent a novel target to combat non–small cell lung cancers. *J Pathol* **212**, 170–179.

[18] Mijatovic T, Van Quaquebeke E, Delest B, Debeir O, Darro F, and Kiss R (2007). Cardiotonic steroids on the road to anti-cancer therapy. *Biochim Biophys Acta* **1776**, 32–57.

[19] Senner V, Schmidtpeter S, Braune S, Püttmann S, Thanos S, Bartsch U, Schachner M, and Paulus W (2003). AMOG/beta2 and glioma invasion: does loss of AMOG make tumour cells run amok? *Neuropathol Appl Neurobiol* **29**, 370–377.

[20] Lefranc F, Mijatovic T, Kondo Y, Sauvage S, Roland I, Debeir O, Krstic D, Vasic V, Gailly P, Kondo S, et al. (2007). Targeting the α_1 subunit of the sodium pump (the Na^+/K^+–ATPase) to combat glioblastoma cells. *Neurosurgery* in press.

[21] Pelloski CE, Lin E, Zhang L, Yung WK, Colman H, Liu JL, Woo SY, Heimberger AB, Suki D, Prados M, et al. (2006). Prognostic associations of activated mitogen-activated protein kinase and Akt pathways in glioblastoma. *Clin Cancer Res* **12**, 3935–3941.

[22] Koul D, Shen R, Bergh S, Sheng X, Shishodia S, Lafortune TA, Lu Y, de Groot JF, Mills GB, and Yung WK (2006). Inhibition of Akt survival pathway by a small-molecule inhibitor in human glioblastoma. *Mol Cancer Ther* **5**, 637–644.

[23] Koul D, Takada Y, Shen R, Aggarwal BB, and Yung WK (2006). PTEN enhances TNF-induced apoptosis through modulation of nuclear factor–kappaB signaling pathway in human glioma cells. *Biochem Biophys Res Commun* **350**, 463–471.

[24] Wiencke JK, Zheng S, Jelluma N, Tihan T, Vandenberg S, Tamgüney T, Baumber R, Parsons R, Lamborn KR, Berger MS, et al. (2007). Methylation

of the PTEN promoter defines low-grade gliomas and secondary glioblastomas. *Neuro Oncol* **9**, 271–279.

[25] Rao RD, Mladek AC, Lamont JD, Goble JM, Erlichman C, James CD, and Sarkaria JN (2005). Disruption of parallel and converging signaling pathways contributes to the synergistic antitumor effects of simultaneous mTOR and EGFR inhibition in GBM cells. *Neoplasia* **7**, 921–929.

[26] Koul D, Shen R, Shishodia S, Takada Y, Bhat KP, Reddy SA, Aggarwal BB, and Yung WK (2007). PTEN down regulates AP-1 and targets c-*fos* in human glioma cells via PI3-kinase/Akt pathway. *Mol Cell Biochem* **300**, 77–87.

[27] Newton HB (2004). Molecular neuro-oncology and development of targeted therapeutic strategies for brain tumors. *Expert Rev Anticancer Ther* **4**, 105–128.

[28] O'Rourke DM (2004). Targeted molecular therapy in glial tumors. *Neurosurgery* **54**, N9 [Letter].

[29] Knobbe CB and Reifenberger G (2003). Genetic alterations and aberrant expression of genes related to the phosphatidylinositol-3-kinase/protein kinase B (Akt) signal transduction pathway in glioblastomas. *Brain Pathol* **13**, 507–518.

[30] Chakravarti A, Zhai G, Suzuki Y, Sarkesh S, Black PM, Muzikansky A, and Loeffler JS (2004). The prognostic significance of phosphatidylinositol-3-kinase pathway activation in human gliomas. *J Clin Oncol* **22**, 1926–1933.

[31] Narita Y, Nagane M, Mishima K, Huang HJ, Furnari FB, and Cavenee WK (2002). Mutant epidermal growth factor receptor signalling down-regulates p27 through activation of the phosphatidylinositol 3-kinase/Akt pathway in glioblastomas. *Cancer Res* **62**, 6764–6769.

[32] Choe G, Horvath S, Cloughesy TF, Crosby K, Seligson D, Palotie A, Inge L, Smith BL, Sawyers CL, and Mischel PS (2003). Analysis of the phosphatidylinositol 3-kinase signalling pathway in glioblastoma patients *in vivo*. *Cancer Res* **63**, 2742–2746.

[33] Joy AM, Beaudry CE, Tran NL, Ponce FA, Holz DR, Demuth T, and Berens ME (2003). Migrating glioma cells activate the PI3-K pathway and display decreased susceptibility to apoptosis. *J Cell Sci* **116**, 4409–4417.

[34] Shingu T, Yamada K, Hara N, Moritake K, Osago H, Terashima M, Uemura T, Yamasaki T, and Tsuchiya M (2003). Synergistic augmentation of antimicrotubule agent–induced cytotoxicity by a phosphoinositide 3-kinase inhibitor in human malignant glioma cells. *Cancer Res* **63**, 4044–4047.

[35] Shingu T, Yamada K, Hara N, Moritake K, Osago H, Terashima M, Uemura T, Yamasaki T, and Tsuchiya M (2003). Growth inhibition of human malignant glioma cells induced by the PI3-K–specific inhibitor. *J Neurosurg* **98**, 154–161.

[36] Nagai S, Washiyama K, Kurimoto M, Takaku A, Endo S, and Kumanishi T (2002). Aberrant nuclear factor–kappaB and its participation in the growth of human malignant astrocytomas. *J Neurosurg* **96**, 909–917.

[37] Aggarwal BB (2004). Nuclear factor–kappaB: the enemy within. *Cancer Cell* **6**, 203–208.

[38] Baldwin AS (2001). Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB. *J Clin Invest* **107**, 241–246.

[39] Sekulic A, Hudson CC, Homme JL, Yin P, Otterness DM, Karnitz LM, and Abraham RT (2000). A direct linkage between the phosphoinositide 3-kinase–AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. *Cancer Res* **60**, 3504–3513.

[40] Hu X, Pandolfi PP, Li Y, Koutcher JA, Rosenblum M, and Holland EC (2005). mTOR promotes survival and astrocytic characteristics induced by Pten/AKT signaling in glioblastoma. *Neoplasia* **7**, 356–368.

[41] Lefranc F, Facchini V, and Kiss R (2007). Pro-autophagic drugs: a novel means to combat apoptosis-resistant cancers. *Oncologist* **12**, 1395–1403.

[42] Kondo Y, Kanzawa T, Sawaya R, and Kondo S (2005). The role of autophagy in cancer development and response to therapy. *Nat Rev Cancer* **5**, 726–734.

[43] Takeuchi H, Kondo Y, Fujiwara K, Kanzawa T, Aoki H, Mills GB, and Kondo S (2005). Synergistic augmentation of rapamycin-induced autophagy in malignant glioma cells by phosphatidylinositol 3-kinase/protein kinase B inhibitors. *Cancer Res* **65**, 3336–3346.

[44] Eshleman JS, Carlson BL, Mladek AC, Kastner BD, Shide KL, and Sarkaria JN (2002). Inhibition of the mammalian target of rapamycin sensitises U87 xenografts to fractionated radiation therapy. *Cancer Res* **62**, 7291–7297.

[45] Groves MD, Puduvalli VK, Chang SM, Conrad CA, Gilbert MR, Tremont-Lukats IW, Liu TJ, Peterson P, Schiff D, Cloughesy TF, et al. (2007). A North American Brain Tumor Consortium (NABTC 99-04) phase II trial of temozolomide plus thalidomide for recurrent glioblastoma multiforme. *J Neurooncol* **81**, 271–277.

[46] Kanzawa T, Bedwell J, Kondo Y, Kondo S, and Germano IM (2003). Inhibition of DNA repair for sensitizing resistant glioma cells to temozolomide. *J Neurosurg* **99**, 1047–1052.

[47] Eoli M, Menghi F, Bruzzone MG, De Simone T, Valletta L, Pollo B, Bissola L, Silvani A, Bianchessi D, D'Incerti L, et al. (2007). Methylation of O^6-methylguanine DNA methyltransferase and loss of heterozygosity on 19q and/or 17p are overlapping features of secondary glioblastomas with prolonged survival. *Clin Cancer Res* **13**, 2606–2613.

[48] Roos WP, Batista LF, Naumann SC, Wick W, Weller M, Menck CF, and Kaina B (2007). Apoptosis in malignant glioma cells triggered by the temozolomide-induced DNA lesion O^6-methylguanine. *Oncogene* **26**, 186–197.

[49] Maiuri MC, Zalckvar E, Kimchi A, and Kroemer G (2007). Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat Rev Mol Cell Biol* **8**, 741–752.

[50] Barnburg JR and Wiggan ONP (2002). ADF/cofilin and actin dynamics in disease. *Trends Cell Biol* **12**, 598–605.

[51] Denker SP and Barber DL (2002). Ion transport proteins anchor and regulate the cytoskeleton. *Curr Opin Cell Biol* **14**, 214–220.

[52] Lefranc F, Yeaton P, Brotchi J, and Kiss R (2006). Cimetidine, an unexpected anti-tumor agent, and its potential for the treatment of glioblastoma [Review]. *Int J Oncol* **28**, 1021–1030.

[53] Lefranc F, James S, Camby I, Gaussin JF, Darro F, Brotchi J, Gabius J, and Kiss R (2005). Combined cimetidine and temozolomide, compared with temozolomide alone: significant increases in survival in nude mice bearing U373 human glioblastoma multiform orthotopic xenografts. *J Neurosurg* **102**, 706–714.

[54] Le Mercier M, Kondo Y, Mathieu V, Camby I, Decaestecker C, Kiss R, and Lefranc F (2007). Decreasing galectin-1 expression in human experimental glioblastomas impairs the endoplasmic reticulum stress (ERS) response and increases temozolomide anti-tumor effects [Abstract]. *AACR* April 2007; accepted for publication.

[55] Camby I, Le Mercier M, Lefranc F, and Kiss R (2006). Galectin-1: a small protein with major functions. *Glycobiology* **16**, 137R–157R.

[56] Megalizzi V, Mathieu V, Mijatovic T, Gailly P, Debeir O, De Neve N, Van Damme M, Bontempi G, Haibe-Kains B, Decaestecker C, et al. (2007). 4-IBP, a sigma1 receptor agonist, decreases the migration of human cancer cells, including glioblastoma cells, *in vitro* and sensitizes them *in vitro* and *in vivo* to cytotoxic insults of proapoptotic and proautophagic drugs. *Neoplasia* **9**, 358–369.

[57] Sjöström M, Stenström K, Eneling K, Zwiller J, Katz AI, Takemori H, and Bertorello AM (2007). SIK1 is part of a cell sodium-sensing network that regulates active sodium transport through a calcium-dependent process. *Proc Natl Acad Sci USA* **104**, 16922–16927.

[58] Xie Z and Askari A (2002). Na(+)/K(+)–ATPase as a signal transducer. *Eur J Biochem* **269**, 2434–2439.

[59] Blanco G (2005). NaK-ATPase subunit heterogeneity as a mechanism for tissue-specific ion regulation. *Semin Nephrol* **25**, 292–303.

[60] Xie Z and Cai T (2003). Na^+-K^+–ATPase–mediated signal transduction: from protein interaction to cellular function. *Mol Interv* **3**, 157–168.

[61] Ye ZC and Sontheimer H (1999). Glioma cells release excitotoxic concentrations of glutamate. *Cancer Res* **59**, 4383–4391.

[62] Takano T, Lin JH, Arcuino G, Gao Q, Yang J, and Nedergaard M (2001). Glutamate release promotes growth of malignant gliomas. *Nat Med* **7**, 1010–1015.

[63] Arcella A, Carpinelli G, Battaglia G, D'Onofrio M, Santoro F, Ngomba RT, Bruno V, Casolini P, Giangaspero F, and Nicoletti F (2005). Pharmacological blockade of group II metabotropic glutamate receptors reduces the growth of glioma cells *in vivo*. *Neuro Oncol* **7**, 236–245.

[64] Munhoz CD, Kawamoto EM, de Sa Lima L, Lepsch LB, Glezer I, Marcourakis T, and Scavone C (2005). Glutamate modulates sodium-potassium–ATPase through cyclic GMP and cyclic GMP-dependent protein kinase in rat striatum. *Cell Biochem Funct* **23**, 115–123.

[65] Stenkwist B (2001). Cardenolides and cancer. *Anticancer Drugs* **12**, 635–636.

[66] McConkey DJ, Lin Y, Nutt LK, Ozel HZ, and Newman RA (2000). Cardiac glycosides stimulate Ca^{2+} increases and apoptosis in androgen-independent, metastatic human prostate adenocarcinomas cells. *Cancer Res* **60**, 3807–3812.

[67] Huang YT, Chueh SC, Teng CM, and Guh JH (2004). Investigation of ouabain-induced anticancer effect in human androgen-independent prostate cancer PC-3 cells. *Biochem Pharmacol* **67**, 727–733.

[68] Mijatovic T, Op De Beeck A, Van Quaquebeke E, Dewelle J, Darro F, de Launoit Y, and Kiss R (2006). The cardenolide UNBS1450 is able to deactivate nuclear factor kappaB–mediated cytoprotective effects in human non–small cell lung cancer cells. *Mol Cancer Ther* **5**, 391–399.

[69] Van Quaquebeke E, Simon G, Andre A, Dewelle J, El Yazidi M, Bruyneel F, Tuti J, Nacoulma O, Guissou P, Decaestecker C, et al. (2005). Identification of a novel cardenolide (2″-oxovoruscharin) from *Calotropis procera* and the hemisynthesis of

novel derivatives displaying potent *in vitro* antitumor activities and high *in vivo* tolerance: structure-activity relationship analyses. *J Med Chem* **48**, 849–856.

[70] Mijatovic T, Lefranc F, Van Quaquebeke E, Van Vynckt F, Darro F, and Kiss R (2007). UNBS1450: a new hemi-synthetic cardenolide with promising anticancer activity. *Drug Dev Res* **68**, 164–173.

[71] Mijatovic T, Mathieu V, Gaussin JF, De Nève N, Ribaucour F, Van Quaquebeke E, Dumont P, Darro F, and Kiss R (2006). Cardenolide-induced lysosomal membrane permeabilization demonstrates therapeutic benefits in experimental human non–small cell lung cancers. *Neoplasia* **8**, 402–412.

[72] Stettner MR, Wang W, Nabors LB, Bharara S, Flynn DC, Grammer JR, Gillespie GY, and Gladson CL (2005). Lyn kinase activity is the predominant cellular SRC kinase activity in glioblastoma tumor cells. *Cancer Res* **65**, 5535–5543.

[73] Gloor S, Antonicek H, Sweadner KJ, Pagliusi S, Frank R, Moos M, and Schachner M (1990). The adhesion molecule on glia (AMOG) is a homologue of the β subunit of the Na,K–ATPase. *J Cell Biol* **110**, 165–174.

[74] Schmalzing G, Kröner S, Schachner M, and Gloor S (1992). The adhesion molecule on glia (AMOG/β_2) and α_1 subunits assemble to functional sodium pumps in *Xenopus* oocytes. *J Biol Chem* **267**, 20212–20216.

[75] Navarro A, Anand-Apte B, and Parat MO (2004). A role for caveolae in cell migration. *FASEB J* **18**, 1801–1811.

[76] Robins HI, Won M, Seiferheld WF, Schultz CJ, Choucair AK, Brachman DG, Demas WF, and Mehta MP (2006). Phase 2 trial of radiation plus high-dose tamoxifen for glioblastoma multiforme: RTOG protocol BR-0021. *Neuro Oncol* **8**, 47–52.

[77] Brandes AA, Nicolardi L, Tosoni A, Gardiman M, Iuzzolino P, Ghimenton C, Reni M, Rotilio A, Sotti G, and Ermani M (2006). Survival following adjuvant PCV or temozolomide for anaplastic astrocytoma. *Neuro Oncol* **8**, 253–260.

[78] Branle F, Lefranc F, Camby I, Jeuken J, Geurts-Moespot A, Sprenger S, Sweep F, Kiss R, and Salmon I (2002). Evaluation of the efficiency of chemotherapy in *in vivo* orthotopic models of human glioma cells with and without 1p19q deletions and in C6 rat orthotopic allografts serving for the evaluation of surgery combined with chemotherapy. *Cancer* **95**, 641–655.

[79] Belot N, Rorive S, Doyen I, Lefranc F, Bruyneel E, Dedecker R, Micik S, Brotchi J, Decaestecker C, Salmon I, et al. (2001). The molecular characterization of cell-substratum attachments in human glial tumors relates to prognostic features. *Glia* **36**, 375–390.

[80] Lefranc F, Sadeghi N, Metens T, Brotchi J, Salmon I, and Kiss R (2003). Characterization of gastrin-induced cytostatic effect on cell proliferation in experimental malignant gliomas. *Neurosurgery* **52**, 881–890.

[81] Molitoris BA, Geerdes A, and McIntosh JR (1991). Dissociation and redistribution of Na^+,K^+–ATPase from its surface membrane actin cytoskeleton complex during cellular ATP depletion. *J Clin Invest* **88**, 462–469.

[82] Lum JJ, DeBerardinis RJ, and Thompson CB (2005). Autophagy in metazoans: cell survival in the land of plenty. *Nat Rev Mol Cell Biol* **6**, 439–448.

[83] Lopez-Lazaro M, Pastor N, Azrak SS, Ayuso MJ, Austin CA, and Cortes F (2005). Digitoxin inhibits the growth of cancer cell lines at concentrations commonly found in cardiac patients. *J Nat Prod* **68**, 1642–1645.