Molecular Mechanisms of Taxol for Induction of Cell Death in Glioblastomas

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Abstract Glioblastomas are the most frequent and devastating brain tumors in adults. Molecular and cytogenetic studies of glioblastomas have revealed a wide variety of deregulated genes that are associated with cell cycles, DNA repair, apoptosis, cell migration, invasion, and angiogenesis with little translational success. Understanding the molecular mechanisms of these deregulated genes can provide a rationale for targeting specific pathways of repair, signaling, and angiogenesis. Taxol, one of the most potent anti-neoplastic drugs, strongly binds to the N-terminal region of β-tubulin to prevent tumor cell division and induce cell death. The effects of taxol may vary depending on cell type and drug concentration. At lower concentrations ranging from 10 to 100 nM, taxol induces phosphorylation of Bcl-2, which in turn triggers mitochondrial release of cytochrome c, cleavage of pro-caspases and poly(ADP-ribose) polymerase (PARP), leading to apoptotic death. Phosphorylation of Bcl-2 also inhibits the ability of Bcl-2 to increase intracellular free [Ca^{2+}], which triggers calpain-mediated apoptosis. At higher concentrations, taxol induces cell death due to stabilization of microtubules and mitochondrial collapse, leading to cell cycle arrest at G2/M phase. Disruption of the mitotic spindle activates a number of signaling pathways, with consequences that may protect the cell. The cells arrested in mitosis exhibit no signal for apoptosis but have an increased expression of survivin, an inhibitor of apoptosis. A thorough understanding of the molecular signaling events associated with taxol-mediated cell cycle arrest is essential, particularly in regard to its potential in combination therapy, where multiple therapeutic agents are used to enhance the efficacy of treatment in controlling cancer cells. In this chapter, we present an overview of the latest research on the molecular signaling mechanisms of taxol, events leading to apoptosis, potential of taxol in combination chemotherapy, and emerging gene therapy.

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Introduction

Malignant brain tumors (malignant gliomas or glioblastomas) are highly invasive and aggressive primary brain tumors and are associated with a dismal prognosis (Pulkkanen and Yla-Herttuala 2005). As per recent American Cancer Society (ACS) statistics (Cancer Facts & Figures, 2008), there would be an estimated 21,810 new cases of primary brain tumors, with an estimated 13,070 deaths in the United States in 2008. Glioblastomas comprise 23% of primary brain tumors and are the most commonly diagnosed brain tumors in both adults and children (Donaldson et al. 2006). Glioblastomas remain highly refractive to therapy, and current treatments produce no long-term survivors in patients with these tumors. The mean survival time of patients with glioblastoma following treatment with surgery, radiotherapy, and chemotherapy is from 9 to 12 months (Castro et al. 2003). Since malignant brain tumor cells often infiltrate deep into the normal brain tissue, complete surgical removal of the brain tumor is almost impossible, retaining the chance of a high incidence of recurrence (Combs et al. 2005). The traditional means of glioblastoma therapy are plagued with numerous side effects and subsequent poor quality of life during the course of treatment. Although understanding of the glioblastoma pathophysiology has increased significantly over the past few years, an effective treatment has not yet been developed for this devastating cancer. Limitations of the current treatment modalities call for the development of novel therapeutic strategies to target the specific biological features for controlling the growth of glioblastomas. The main focus of this chapter is the description of current understandings of the molecular mechanisms of taxol for induction of apoptosis in glioblastoma.

Causes of Brain Tumors

Generally, cancers are associated with one or more risk factors, but the only known environmental risk factor for brain tumor is ionizing radiation (Lü et al. 2006; Fisher et al. 2007). People receiving radiotherapy (high-dose ionizing radiation) to the head during childhood are at increased risk for developing brain tumors. Primary brain tumors may also result from specific genetic diseases or cancer-causing chemicals such as vinyl chloride, N-nitroso compounds and polycyclic aromatic hydrocarbons (Lewis et al. 2003; Schwartzbaum et al. 2006). Brain tumors sometimes occur in several members of the same family, which suggests the involvement of some genetic influence. Nevertheless, the exact causes of most primary brain tumors remain a mystery. It is clear that primary brain tumors are not contagious. There is no relation between primary brain tumors and smoking (Zheng et al. 2001), diet (Huncharek et al. 2003), use of cellular phones (Takebayashi et al. 2008),

or electromagnetic fields (Connelly and Malkin 2007). Brain tumors occur more often among white people than among people of other races (Barnholtz-Sloan et al. 2003). A single-cell gene mutation or deletion of a tumor suppressor gene may trigger an abnormal cell division, which finally forms an intracranial tumor. The risk of developing brain cancer increases with age. In the United States, the rate of occurrence of brain cancer in people under age 65 is 4.5 per 100,000 people, whereas the rate in people at age 65 and older is 17.8. Patients with a history of metastatic cancers such as melanoma, lung, breast, colon, and kidney cancers are at high risk for secondary brain tumors.

Treatments for Glioblastomas

Glioblastomas are difficult to treat because of their location in the brain. Moreover, malignant brain tumor cells are highly resistant to chemotherapy and other conventional therapies. Current treatment regimens – surgery, chemotherapy, and radiation – cannot cure any brain tumor. Since the highly invasive glioblastoma cells penetrate deep inside normal tissue, it is practically impossible to remove the tumors completely through surgery. Almost all glioblastomas will recur within three months after surgery. Many chemotherapeutic agents may not be effective for brain tumors because they do not pass through the blood brain barrier (BBB) effectively (Bellavance et al. 2008). Additionally, chemotherapy has numerous undesirable side effects, resulting in poor quality of life for the patients. Radiation therapy for glioblastomas also affects normal cells adversely, causing severe side effects to the patient and further inducing the formation of primary brain tumors. However, recent advances in microsurgery techniques, radiotherapy, and chemotherapy are slowly increasing the survival time of patients diagnosed with glioblastoma. Here we elaborate only on chemotherapy, with special emphasis on taxol and its molecular mechanisms of action for induction of cell death in glioblastomas.

Chemotherapy for Glioblastomas

Chemotherapy is moderately effective in controlling the growth of glioblastomas. At present, several different types of chemotherapeutic agents are available for the treatment of glioblastomas. However, most of the chemotherapeutic agents for glioblastomas result in serious clinical problems that adversely affect both the quality of life and the ability of patients to continue treatment. Generally, chemotherapy for primary brain tumors begins only after surgery and radiation. Almost all cancer chemotherapeutic agents are based on the principle of impairing mitosis, effectively targeting fast-dividing cells. Although chemotherapy is targeted against fast-dividing tumor cells, it also affects normal cell division and may lead to several side effects. The specific features of tumor cells that make them uniquely targetable to chemotherapeutic

agents have yet to be identified. Specifically, the normal cells that can be affected with chemotherapy are those in the bone marrow and the cells that line the gastrointestinal tract. Chemotherapy may also affect both male and female gametogenesis and can produce defective sperm or ovum (Thomson et al. 2002). Certain chemotherapeutic agents may induce permanent sterility in males. Furthermore, many chemotherapeutic agents are also neurotoxic, nephrotoxic, and hepatotoxic. The toxicity of the anti-cancer agents also arises from the solvents used to dissolve these agents (Zhang et al. 2005). Another drawback of chemotherapy is the development of drug-resistant cells within the tumors and inadequate drug delivery into the brain due to the presence of the BBB. Temodar (temozolomide) and taxol (paclitaxel) are two highly promising chemotherapeutic agents that are currently being evaluated for the treatment of glioblastomas. It has been demonstrated that temozolomide is more effective and powerful than taxol for the treatment of malignant brain tumors. Since temozolomide is an alkylating agent, it demethylates the promotor region of the gene for O-6-methylguanine-DNA methyltransferase (MGMT), an important DNA repair enzyme that removes methyl adducts at the O-6-position of guanine, one of the most prominent and biologically important targets of alkylating agents. MGMT function is frequently lost due to the hypermethylation of CpG islands in the promoter region of this enzyme in many types of human anaplastic astrocytomas, including glioblastomas (Esteller et al. 1999). Temozolomide treatment has further advantages because several other important genes involved in cell cycle regulation and apoptosis are also silenced in malignant gliomas due to hypermethylation of their promoter CpG islands (Konduri et al. 2003; Ohta et al. 2006; Martinez et al. 2007).

Taxol

Taxol (paclitaxel) was first isolated from the bark of the Pacific yew tree *Taxus brevifolia* (Wani et al. 1971) at the Research Triangle Institute, North Carolina, USA. Fig. 1 illustrates the molecular structure of taxol. In 1979, Susan B. Horwitz and her group at Albert Einstein College of Medicine, Bronx, New York, showed that the mechanism of the action of taxol involves the stabilization of microtubules (Schiff et al. 1979). Robert A. Holton and his group at Florida State University first succeeded in the total synthesis of taxol in 1994 (Holton et al. 1994a, b). Taxol strongly binds to the N-terminal region of the β-subunit of tubulin and promotes the formation of highly stable microtubules that resist depolymerization, thus preventing active tumor cell division and arresting the cell cycle at the G2/M phase (Schiff and Horwitz 1980; Ganesh et al. 2007). The arrest of microtubules inhibits the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitosis. Even though it has been well established that taxol inhibits cell division through mitotic arrest, it is unclear whether taxol-induced cell death also represents a secondary event resulting from mitotic arrest or involves a novel mechanism of action.

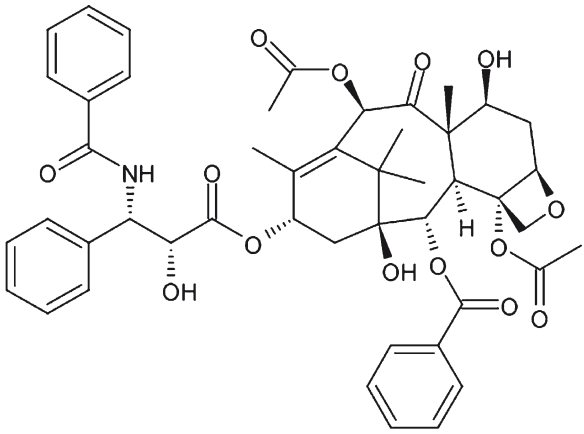

Fig. 1 Chemical structure of taxol ($C_{47}H_{51}NO_{14}$; MW 853.91)

Molecular Mechanisms of Taxol-Induced Cell Death

Mechanism of Cell Cycle Arrest

The exact mechanism of taxol for cytotoxicity against tumor cells is not entirely clear. Unlike classical anti-microtubule agents, such as colchicine and vinblastine that induce microtubule disassembly and/or paracrystal formation (Wilson et al. 1974), taxol inhibits microtubule depolymerization and promotes the formation of highly stable microtubules, thereby disrupting the normal dynamic reorganization of the microtubule network required for mitosis and cell proliferation (Baum et al. 1981; Manfredi and Horwitz 1984). Tubulin is a member of the family of globular proteins, which mainly includes α-tubulin and β-tubulin. Microtubules are polymers assembled from dimers of α-tubulin and β-tubulin. During polymerization, the heterodimer formed from α-tubulin and β-tubulin binds to two molecules of guanosine triphosphate (GTP): a non-exchangeable GTP molecule at the α-subunit, which plays a structural stability role (Menéndez et al. 1998); and an exchangeable GTP molecule bound to the β-subunit. Upon assembly of the α-tubulin/β-tubulin heterodimer, GTP bound to β-tubulin is hydrolyzed to GDP, reaching a steady-state equilibrium between free tubulin dimers and microtubules (Xiao et al. 2006). In the GDP-bound state, the protein is in an inactive conformation, forming double rings (Díaz et al. 1994), whereas in the GTP state, it is active for microtubule assembly. The GTP-GDP hydrolysis in the heterodimers controls the assembled state of tubulin (Symmons et al. 1996). Taxol drives inactive GDP-tubulin into microtubules, replacing the need of the γ-phosphate of GTP to activate the protein (Díaz and Andreu 1993). Taxol stabilizes microtubules by binding preferentially to assemble tubulin with an exact 1:1 stoichiometric ratio (Díaz et al. 2000). Unpolymerized tubulin has no significant affinity for taxol (Díaz et al. 2000), indicating that the binding site is formed during the polymerization process.

Taxol is highly effective in inducing cell cycle arrest in human glioblastoma cells. We treated human glioblastoma U251MG cells (obtained from the National Cancer Institute, Frederick, MD) with 100 nM taxol (Bristol-Myers Squibb, Princeton, NJ) for 48 h in culture and analyzed them by a fluorescent activated cell sorting (FACS) system. Figure 2 shows the FACS histogram of U251MG cells after treatment with 100 nM taxol. In untreated U251MG culture, 75% of cells were in the G1 phase and 6% were in the G2/M phase, leaving 19% of cells in the S phase. However, after taxol treatment, 75% of cells were arrested in the G2/M phase, leaving 18% in the G1 phase and only 7% in the S phase. Since most of the apoptotic/dead cells were lost during processing for FACS analysis, the percentage of apoptotic cells after taxol treatment was lower compared to our previous report (George et al. 2009).

Mechanisms of Taxol for Induction of Apoptosis

Apoptosis, the terminal point of programmed cell death, is well characterized by morphological and biochemical features (Waring et al. 1991). Several lines of evidence from recent studies have suggested that taxol induces apoptosis through a signaling mechanism independent of microtubule and mitotic arrest (Fan 1999; Impens et al. 2008). Dziadyk et al. showed that taxol induced apoptosis due to

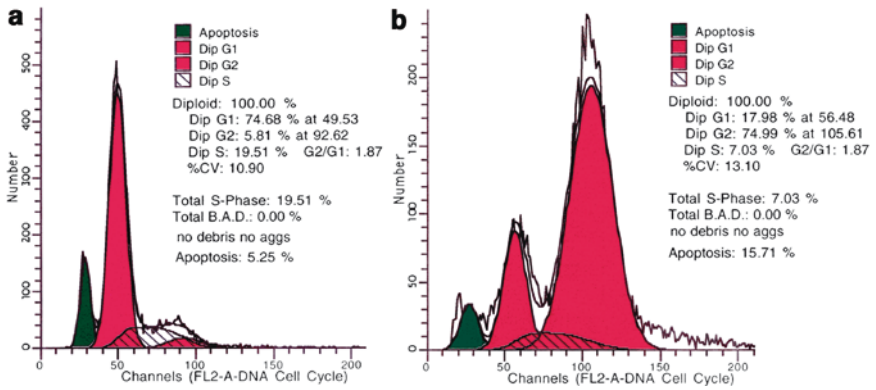

Fig. 2 Determination of cell cycle arrest in human glioblastoma U251MG cells after treatment with taxol. FACS histogram of cells after treatment with 100 nM taxol for 48 h. Untreated and treated cells (in 6-well culture plates) were harvested using TrypLE (Invitrogen, Carlsbad, CA) after washing twice with serum-free media. The cells were centrifuged, washed again with PBS, dispersed in 1 ml of propidium iodide (50 μg/ml) (Biosure, Grass Valley, CA) with gentle vortex, and then incubated for 30 min in the dark at 4°C. The cells were sorted on a FACS machine (FACSCalibur, Becton and Dickinson, Franklin Lakes, NJ) based on the red fluorescence at 488 nm. (**a**) Untreated cells showing 75% of cells in G1 phase. (**b**) Cells after taxol treatment showing 75% of cells arrested at G2/M phase. Most of the apoptotic/dead cells were lost during washing. Data are representative of 5 independent experiments

modulation of the NF-κB/IκB signaling pathway (Dziadyk et al. 2004). The c-Jun N-terminal kinase (JNK) signaling pathway also plays an important role in taxol-mediated apoptosis (Vivat-Hannah et al. 2001; Mingo-Sion et al. 2004). It is now well established that taxol triggers apoptosis by both caspase-dependent (Day et al. 2006; Janssen et al. 2007; Pineiro et al. 2007) and caspase-independent pathways (Huisman et al. 2002; Broker et al. 2004).

Treatment of glioblastoma cells with taxol could activate the cysteine proteases of the calpain and caspase families. We treated human glioblastoma U251MG cells with 100 nM taxol for 72 h and stained for m-calpain (calpain-2) and an active subunit (cleaved fragment) of caspase-3 using double immunofluorescent staining (Fig. 3). There was a marked upregulation in the expression of both m-calpain and the active subunit of caspase-3 in apoptotic cells. The upregulation of m-calpain and the active subunit of caspase-3 after taxol treatment indicated that taxol triggered caspase-dependent apoptotic signaling pathways. It has been demonstrated that taxol treatment upregulates tumor necrosis factor-α (TNF-α) (Ding et al. 1990) and tumor necrosis factor-related apoptosis inducing ligand (TRAIL) (Nimmanapalli et al. 2001). In one of our experiments (George et al. 2009), we have shown an increase of intracellular free [Ca^{2+}] after taxol treatment in U251MG cells in culture. An increase in intracellular free [Ca^{2+}] can upregulate m-calpain, which in turn triggers a caspase-mediated pathway for apoptosis. The binding of taxol to microtubules exerts endoplasmic reticulum (ER) stress, which causes release of free Ca^{2+} into the cytoplasm. Taxol also phosphorylates Bcl-2 to eliminate its anti-apoptotic effect, which accelerates the release of cytochrome c from mitochondria into the cytosol (Leung et al. 2000; Figueroa-Masot et al. 2001) and initiates the formation of an apoptosome along with apoptotic protease-activating factor-1 (Apaf-1) in the presence of adenosine nucleotides (Liu et al. 1996). In non-neuronal cells, taxol-induced apoptosis requires activation of JNK that phosphorylates and inactivates Bcl-2. Figure 4 shows a schematic representation of the molecular mechanisms of taxol triggering both caspase-dependent and caspase-independent signaling pathways for induction of apoptosis. We have also demonstrated increased cleavage of DNA fragmentation factor-45 (DFF-45) and poly (ADP-ribose) polymerase (PARP) in the course of apoptosis in U251MG cells in culture after treatment with taxol (George et al. 2009).

Formulation of Nanotaxol

Several attempts to formulate nanotaxol have been undertaken to deliver treatment more efficiently for various cancers, including glioblastomas (Nikanjam et al. 2007; Trickler et al. 2008). However, none of the available techniques efficiently delivers taxol via BBB into the brain. Recently, Eugene R. Zubarev and his group in Rice University, Texas, discovered a method to load dozens of molecules of taxol onto tiny gold spheres many times smaller than living cells (Gibson et al. 2007). Figure 5 demonstrates the formulation of nanotaxol, where several molecules of taxol are

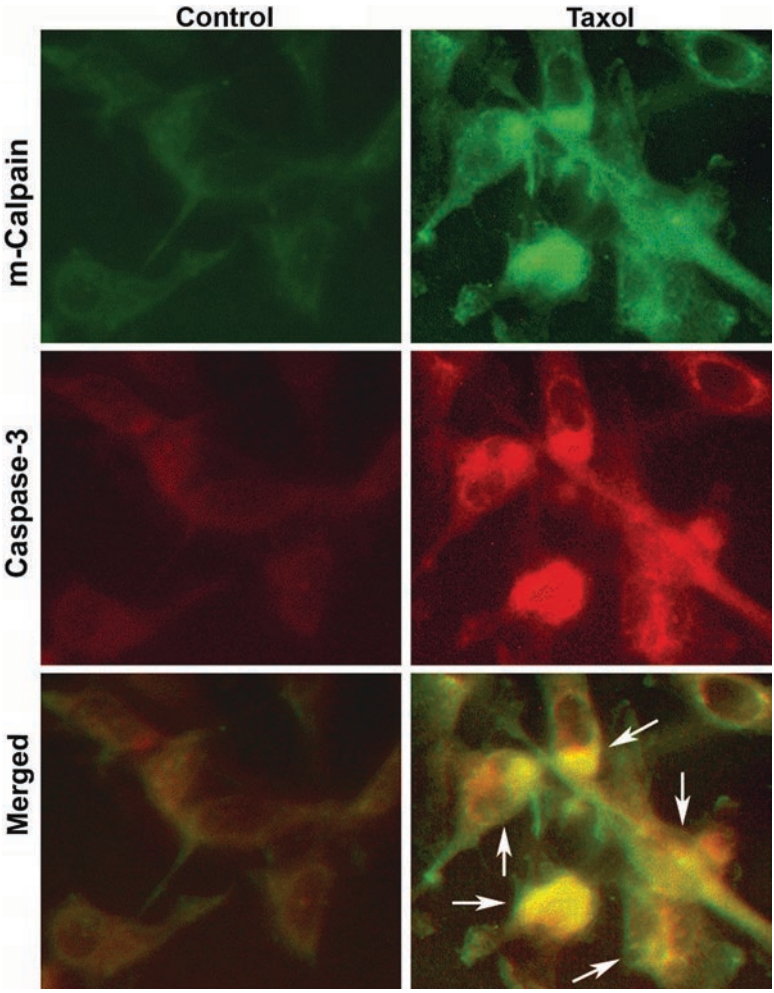

Fig. 3 Double immunofluorescent stainings to examine the levels of the expression of m-calpain and active caspase-3 in human glioblastoma U251MG cells after treatment with 100 nM taxol for 72 h. The cells cultured on chamber slides were fixed with 95% ethanol and blocked with 2% goat and 2% donkey sera (50:50) in PBS for 1 h. The cells were washed and treated with rabbit polyclonal m-calpain (Cell Signaling Technology, Danvers, MA) and goat polyclonal active caspase-3 (Santa Cruz Biotechnology, Santa Cruz, CA) primary IgG antibodies simultaneously and incubated overnight at 4°C. The cells were washed and incubated with FITC-conjugated goat anti-rabbit and Texas red-conjugated donkey anti-goat secondary IgG antibodies (Biomeda, Foster City, CA) at room temperature for 1 h for the detection of m-calpain and caspase-3, respectively. Electronic merging of the stained images demonstrated the simultaneous expression and co-localization of m-calpain and active caspase-3 in apoptotic cells. Arrows indicate apoptotic cells

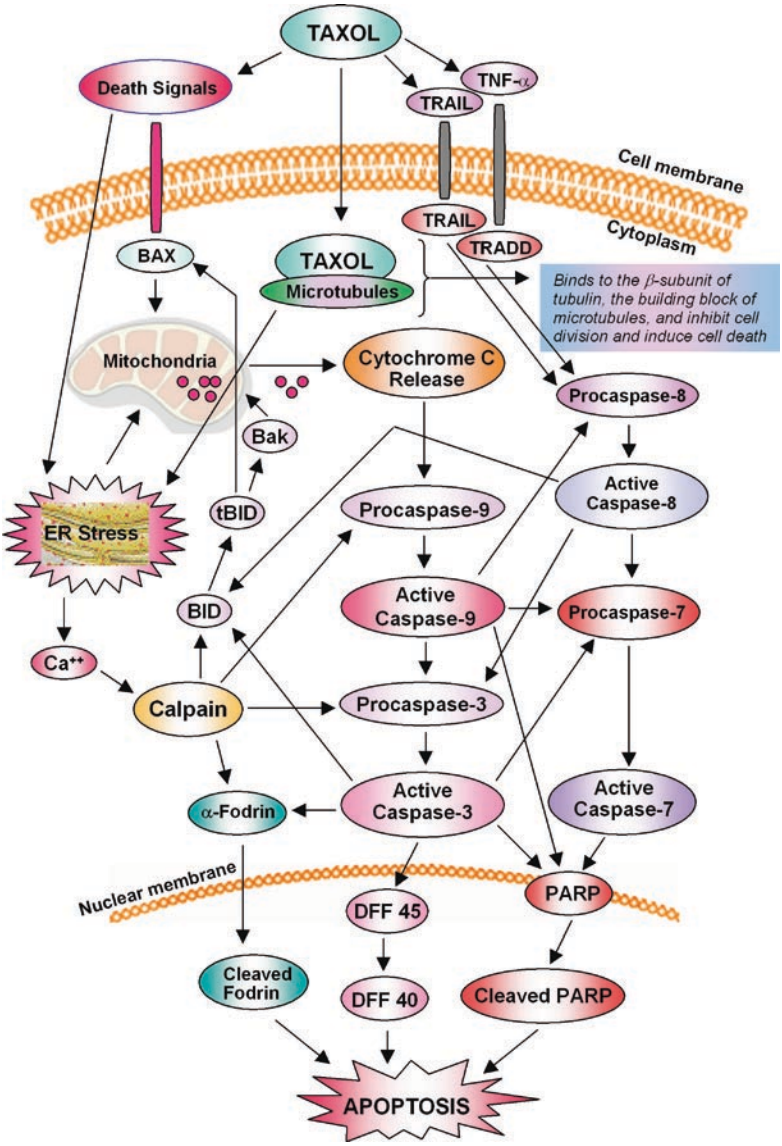

Fig. 4 Molecular mechanisms of taxol for the induction of cell death in glioblastomas. Taxol strongly binds to the β-subunit of tubulin and promotes the formation of highly stable microtubules, which results in cell cycle arrest at the G2/M phase and induces cell death. Taxol also triggers death signals that cause ER stress and upregulation of Bax. Taxol binding to the microtubules also leads to ER stress and increases intracellular free [Ca^{2+}] that upregulates calpain. Furthermore, taxol upregulates tumor necrosis factor-α (TNF-α) and TRAIL, which trigger the extrinsic caspase pathway through TNF receptor-1-associated death domain (TRADD). Bcl-2 phosphorylation accelerates the mitochondrial release of cytochrome c into the cytosol. The association of cytosolic cytochrome c with pro-caspase-9 and Apaf-1 processes pro-caspase-9 to its active form, which then triggers the intrinsic pathway of apoptosis. Pro-caspase-3 is cleaved to its active form by calpain, caspase-9, and caspase-8. The active caspase-3 in turn cleaves α-fodrin, DFF45, and PARP, leading to DNA fragmentation and apoptosis

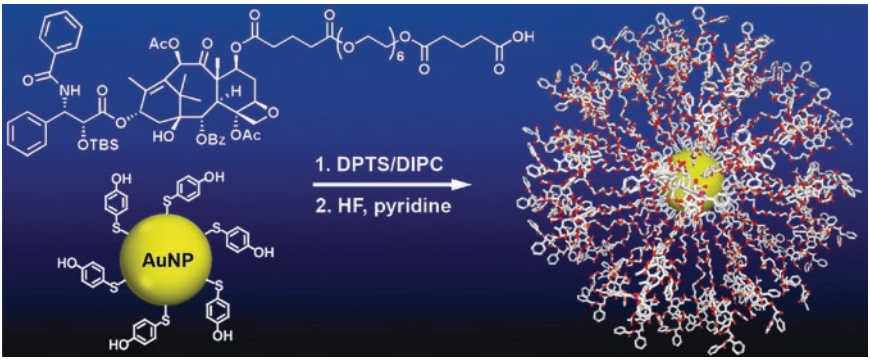

Fig. 5 Formulation of nanotaxol. The anti-cancer drug taxol is covalently functionalized with 2 nm gold nanoparticles (AuNPs). The synthetic strategy involves the attachment of a flexible hexaethylene glycol linker at the C-7 position of taxol followed by coupling of the resulting linear analog to phenol-terminated gold nanocrystals. (Reproduced with permission from Professor Eugene R. Zubarev, Department of Chemistry, Rice University, Texas)

covalently functionalized with 2 nm gold nanoparticles (AuNP). Here a flexible hexaethylene glycol linker is attached to the taxol at the C-7 position, and the resulting linear analog is coupled to phenol-terminated gold nanocrystals. There are about 70 molecules of taxol covalently linked per 1 gold nanoparticle. This technique provides a rare opportunity to prepare hybrid particles with a well-defined amount of taxol and offers a new alternative for the design of nanosized drug-delivery systems. This approach also allows a more accurate measurement of therapeutic activity as a result of the increased ability to quantify the amount of drug present. The use of nanotaxol to deliver the drug via BBB can help successful treatment of glioblastomas.

Molecular Gene Therapy for Glioblastomas

Gene therapy involves the use of nucleic acids, which may include DNA and RNA for treatment of diseases. Gene therapy could modify the genetic composition of the target cells, which is not possible to achieve with any other treatment modality. This novel therapeutic strategy can also be used in combination with traditional treatment techniques to prolong the lifespan of patients and, ultimately, control and/or cure brain tumors. Several novel, more efficient, and less toxic molecular techniques are under development: for example, mammalian expression and viral vectors to deliver small interfering RNAs (siRNAs) for silencing oncogenes and anti-apoptotic molecules; and putative therapeutic gene delivery into the central nervous system (CNS). Such advances would constitute a new treatment paradigm and alternative modalities to control devastating glioblastomas. Since the traditional treatment strategies for glioblastomas and many other cancers are not effective

and are plagued with undesirable side effects, gene therapy is a particularly promising approach for the treatment of these cancers. As we now have a better understanding of the pathophysiology of glioblastomas, including the mechanisms of tumor invasion and angiogenesis, it is possible to target the pathogenetic oncogenes or reactivate the silenced tumor suppressor genes through the delivery of functional genes or nucleotide sequences via efficient synthetic vectors carrying powerful promoters. A large number of mammalian expression vectors carrying the gene for coral green fluorescent protein (cGFP) and luciferase are available for the efficient monitoring of gene delivery, both in vitro and in vivo. With the introduction of effective and powerful non-invasive animal imaging systems (eg: Xenogen, Bioscan), it is possible to monitor the regression of tumors in experimental animals following the successful delivery of mammalian expression or viral vectors carrying the nucleotide sequences or the gene of interest. With the advent of positron emission tomography (PET) in conjunction with single photon emission computed tomography (SPECT), it could be possible to track the delivery of plasmid and viral vectors into human organs or tissues for various gene therapeutic applications. Tumor cells evade immunosurveillance through active participation in inducing tumor-specific immunosuppression, which facilitates easy entrance of plasmid and viral vectors into tumor cells. However, the high level of heterogeneity that exists among tumor cells may present significant challenges to the uniform delivery of such vectors into cancer cells.

Gene therapy for cancer mainly involves either expression of the silenced tumor suppressor genes (e.g., p53, PTEN) or suppression of the oncogenes (e.g., Ras, c-Myc). Gene therapy also involves silencing of several molecules that promote tumor cell invasion (e.g., MMP-9) and angiogenesis (e,g., VEGF) through antisense oligonucleotides or through mammalian expression plasmid vectors carrying the cDNA for specific siRNAs. Furthermore, gene therapy is used to express several important genes that are silenced in tumor cells due to hypermethylation of their promoter regions (George et al. 2007; Sasai et al. 2007). However, expression or suppression of a single gene or protein will do little to help in the treatment of glioblastomas or any other type of cancer. Gene therapy must therefore include simultaneous use of several genes/molecules involved in the pathogenesis of cancers in conjunction with traditional therapeutic approaches. Unfortunately, there are only few reports of combining gene therapy with conventional treatment modalities. The combination of synthetic Bcl-2 siRNA and a low-dose of cisplatin resulted in a massive induction of apoptotic death, with almost complete suppression of cell growth in malignant melanoma (Wacheck et al. 2003). Plasmid vectors expressing Bcl-2 and Bcl-xL shRNAs sensitize human hepatoblastoma cells to the chemotherapeutic drugs 5-fluorouracil and 10-hydroxycamptothecin (Lei et al. 2007). These results suggested that Bcl-2 and Bcl-xL siRNA-mediated gene silencing in combination with chemotherapy would be a potential therapeutic strategy against human hepatoblastoma (Lei et al. 2007). A patient with glioblastoma who is alive and disease-free 13 years following aggressive treatment with multiple surgeries, radiotherapy, chemotherapy, and gene therapy has been reported recently (Karabatsou and Bernstein 2008).

Studies in our laboratory demonstrated that sequential intraperitoneal administrations of mammalian expression plasmid vector carrying Bcl-xL siRNA cDNA (50 μg DNA/injection/mouse) in conjunction with a low dose of taxol (50 μg/injection/mouse) resulted in marked regression of intracranial tumorigenesis in nude mice (Fig. 6). Even though the treatments with either Bcl-xL siRNA or taxol resulted in significant reduction of tumor volume compared with the scrambled Bcl-xL siRNA-treated animals, the treatment with a combination of both agents resulted in a synergistic effect. We also observed complete inhibition of in vivo angiogenesis (dorsal skinfold chamber model) and remarkable regression of both intracranial and subcutaneous tumorigenesis in nude mice after treatment with a combination of taxol and Bcl-2 siRNA (George et al. 2009).

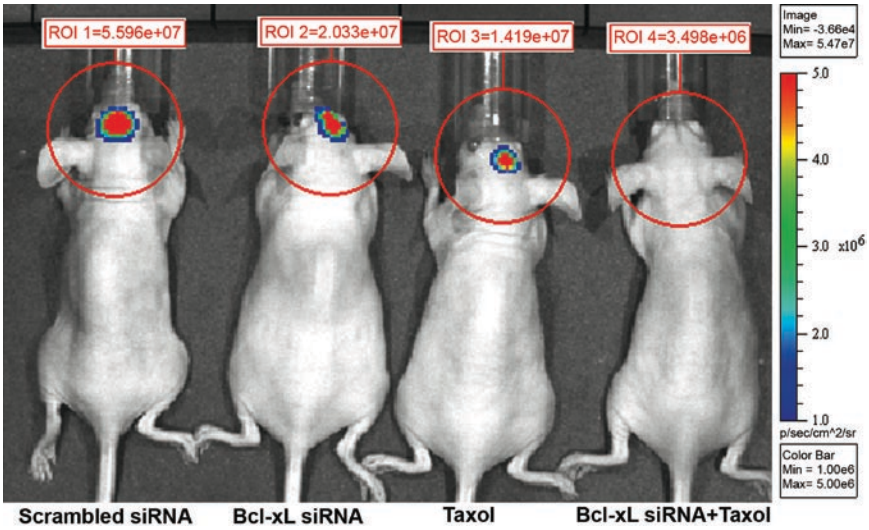

Fig. 6 Synergistic effect of Bcl-xL siRNA and taxol in the inhibition of intracranial tumor in nude mice. Human glioblastoma U251MG cells were stably transfected with a mammalian expression vector (phCMV-FSR, Genlantis, San Diego, CA) carrying the luciferase gene and propagated in media containing G-418 (Mediatech, Manassas, VA) at a concentration of 500 μg/ml. About 1×10^6 cells suspended in 10 μl of serum-free media were injected intracranially with the help of a stereotactic instrument after drilling a small hole in the cranium of the nude mouse. Beginning from day 3 after implantation of the tumor cells, the mice were injected intraperitoneally with either a mammalian expression vector carrying Bcl-xL siRNA cDNA (pRNAT-CMV3.2/Neo, GenScript, Piscataway, NJ) (50 μg DNA/injection/mouse) or taxol (50 μg/injection/mouse), or both agents together for 28 days on alternate days. On day 30, the mice were injected with 100 μl of luciferin (Genlantis, San Diego, CA) at a concentration of 50 mg/ml. After 10 min, the mice were visualized for luciferase activity using the Xenogen IVIS-200 (Xenogen, Hopkinton, MA) imaging system. The combination treatment with Bcl-xL siRNA and taxol resulted in complete inhibition of intracranial tumorigenesis in nude mice. The data are representative of four sets of animals in each group. The absence of a visible tumor image in the mouse after treatment with combination of Bcl-xL siRNA and taxol does not mean that the tumor is regressed completely. A very small tumor was still inside the intracerebral region of the brain, as indicated by the number of photons (3.498e+06) during in vivo imaging. The background signal from a normal mouse is about 1.5e+05 photons on a Xenogen IVIS-200 imaging machine

Previous studies demonstrated that intraperitoneal administration of taxol is more effective than intravenous administration both in patients and in experimental animals (Hribaschek et al. 2007; Tsai et al. 2007). Our studies along with the previous reports indicate that the intraperitoneal route is appropriate for gene therapy in conjunction with anti-cancer drugs. However, the requirement of a large amount of substances is a rate-limiting factor.

Even though many different anti-cancer gene therapy approaches are being developed, it is unlikely that any of these strategies would effectively treat or cure cancer. Gene therapy will likely be successful when several different strategies are used in combination. Furthermore, gene therapy should be used in conjunction with traditional cancer therapeutic approaches, such as surgery, chemotherapy, and radiation. Along with recent advances in conventional cancer treatment modalities and effective non-invasive imaging systems such as PET, gene therapy is likely to be a promising tool for the effective treatment and cure of devastating cancers, including glioblastomas.

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