Chapter 12

Current Applications of 5-ALA in Glioma Diagnostics and Therapy

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1. Introduction

Gliomas, the most common primary brain tumors, are characterized by rapid proliferation, marked infiltration, and poor prognosis and are known for their dismal outcomes (Iacob & Dinca, 2009; Lefranc et al., 2006). The infiltrative nature of malignant glioma makes complete resection difficult, as tumor margins are unclear. Recurrence of glioma takes place within approximately 2 cm of the margins of the resected cavity owing to its invasive character (Aydin et al., 2001; Wallner et al., 1989). The use of fluorescence to delineate tumor margins intraoperatively has emerged as a safe and effective tool for increasing the extent of resection. Therefore, methods that easily detect tumor margins during surgery would be extremely beneficial. 5-aminolevulinic acid (5-ALA) fluorescence-guided glioma resection is a rapidly growing, novel approach to improve the extent of tumor resection with broad applications in both preclinical and clinical settings (Stummer et al., 2000; Stummer et al., 1998b; Stummer et al., 1998c). Intraoperative tumor fluorescence provided by the chemical compound 5-ALA assists surgeons in identifying the true tumor margin during resection of glial neoplasms, consequently increasing the extent of the resection. 5-ALA is the most studied fluorescer and has been used in many clinical trials, including a multicenter phase III randomized controlled trial. Recent controlled Phase III clinical trials have demonstrated that this surgical method enables more complete resection of contrast-enhancing lesions than conventional microsurgery and improves progression-free survival in patients with malignant glioma (Pichlmeier et al., 2008; Stummer et al., 2006).

Photodynamic therapy (PDT) is a treatment modality that takes advantage of the cytotoxic effects induced by a photosensitizer and light in the presence of oxygen (Norum et al., 2009). This

photodamage may be due to direct cytotoxicity, vascular damage, and inflammatory and immunological responses (Castano et al., 2006). The relative importance of these pathways to the therapeutic effects depends on the tissue oxygenation, photosensitizer formulation, distribution, and light dosimeter (Norum et al., 2009). 5-ALA-induced protoporphyrin IX (PpIX) can also be used for 5-ALA-PDT rather than photodynamic diagnosis (PDD) by exposing the field to either blue or red laser light; however, a red light at 632 nm would target a much larger tumor volume owing to better light penetration (Eljamel et al., 2008). Activation of PpIX by red light in combination with oxygen causes cell death by apoptosis and necrosis through the release of cytotoxic singlet oxygen (Peng et al., 1997). This tool may aid in overcoming the hurdle of residual tumor cells after traditional neurosurgery. Application of 5-ALA-PDT has been emerging as a new field and is expected to create a major breakthrough in addressing several unsolved medical issues, especially photokilling of residual neoplastic cells.

Developing technologies such as fluorescence operation-systems to enhance endogenous fluorescence has fairly recently been shown to delineate brain tumor margins intraoperatively (Babu & Adamson, 2012). 5-ALA fluorescence-guided resection shows great promise for furthering our surgical abilities and will become the standard of care for patients diagnosed with malignant glioma in the foreseeable future. The following review analyzes the recent literature in an effort to describe how these modalities involving 5-ALA in glioma diagnostics and therapy can and should be used in the treatment of patients with glioma. This article reviews recent developments in the use of 5-ALA for simultaneous imaging and 5-ALA-triggered photodamage in glioma patients (Figure 1).

2. General knowledge of 5–ALA

5-ALA (molecular weight, 167.6) is a natural biochemical precursor for heme synthesis in living mammalian cells (Eljamel et al., 2008; Peng et al., 1997). Each cell metabolizes 5-ALA along a set pathway toward heme production, inducing the synthesis of the endogenous fluorescent molecule, PpIX, through metabolic conversion in the mitochondria (Bottomley & Muller-Eberhard, 1988). When PpIX emits peak fluorescence at 635 nm with a peak excitation wavelength at 405 nm, it is observed as a red light through a filter that allows this wavelength to pass. Clinical use of 5-ALA for PDT or PDD has also been reported in dermatology, urology, neurosurgery, otorhinolaryngology, gynecology, and gastroenterology for various epithelia and cancerous tissues (D'Hallewin et al., 1998; Guyon et al., 2012; Loning et al., 2004; Piotrowski et al., 2004). In the USA, it has been used with the approval of the Food and Drug Administration (FDA) as a therapeutic drug for solar keratosis. Its use has not yet been approved by the pharmaceutical authority in Japan, and it is only used after obtaining approval from the ethics committee of the respective institutes. The tumor tissue concentration of the fluorescent dye peaks at 2–6 hours after oral administration and disappears by 12 hours. Administration of antacid should be avoided at the time of oral administration because the dye is easily decomposed in the presence of alkali, and use of 5-ALA is contraindicated in cases of porphyria, a genetic disease. PpIX biosynthesized from 5-ALA is a phototoxic substance, although the incidence of skin photosensitivity is lower than that re-

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ported for conventional porphyrin derivatives (Stummer et al., 2006; Toda, 2008). When given orally, 5-ALA has been shown to produce fluorescence in glial neoplasms both *in vitro* and *in vivo* (Blake & Curnow, 2010). In addition, the fluorescence of PpIX is itself cytotoxic and has the potential for use as an adjuvant photodynamic therapy for neoplastic tissue that cannot be safely resected (Sherman et al., 2011). Since the administered 5-ALA is excreted into the urine within 24 hours after oral administration and does not remain in the skin, the occurrence of photosensitivity can be adequately prevented by avoiding sun exposure for approximately 24 hours after administration.

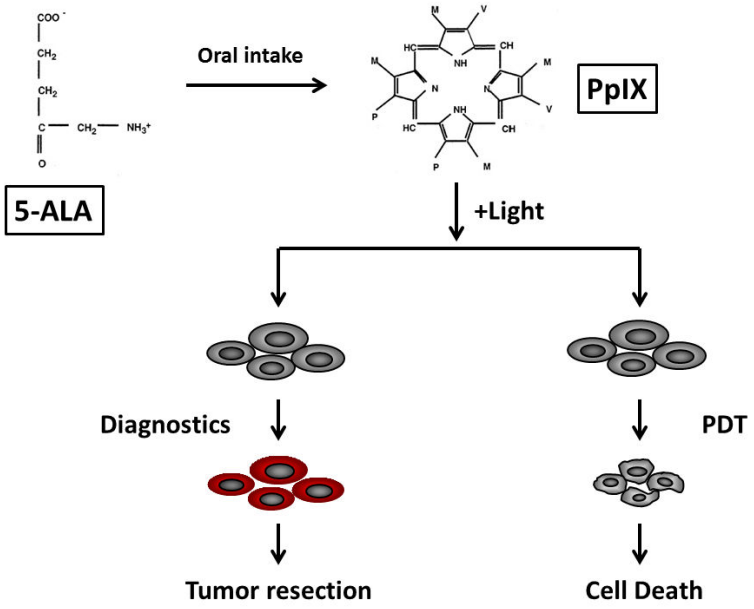

Figure 1. Schematic representation of diagnostics and PDT of 5-ALA for glioma cells. The figure illustrates 5-ALA is converted to PpIX in malignant gliomas via an oral-intake of exogenous 5-ALA. In the presence of an appropriate light source with the specific wavelength, PpIX fluorescence acts bimodal function; fluorescence diagnostic marker and 5-ALA-PDT. Red fluorescence acts as a discriminating marker to assist neurosurgeons to visualize the extent and margins of tumors; 5-ALA-PDT results in a series of irreversible photochemical and photobiological events that cause directly damage and killing glioma cells.

3. 5–ALA biology in glioma

5-ALA, the metabolic precursor of heme in the heme biosynthesis pathway, is not itself fluorescent, but is metabolized into endogenous fluorescent PpIX (Gossner et al., 1998; Inoue et al., 2007; Kennedy & Pottier, 1992; Loh et al., 1993; Peng et al., 1997). Heme biosynthesis consists of a series of enzyme-catalyzed steps, involving 5-aminolevulinate synthases 1 and 2,

5-aminolevulinate dehydratase, hydroxymethylbilane synthase, uroporphyrinogen III synthase, uroporphyrinogen decarboxylase, coproporphyrinogen oxidase (CPOX), protoporphyrinogen oxidase, and ferrochelatase (FECH). In addition to these enzymes, several transporters are also involved in the biosynthesis and catabolism of heme and porphyrin, including oligopeptide transporters 1 and 2 (PEPT1 and PEPT2) and the ABC transporters ABCB6 and ABCG2 (Takahashi et al., 2011) (Figure 2).

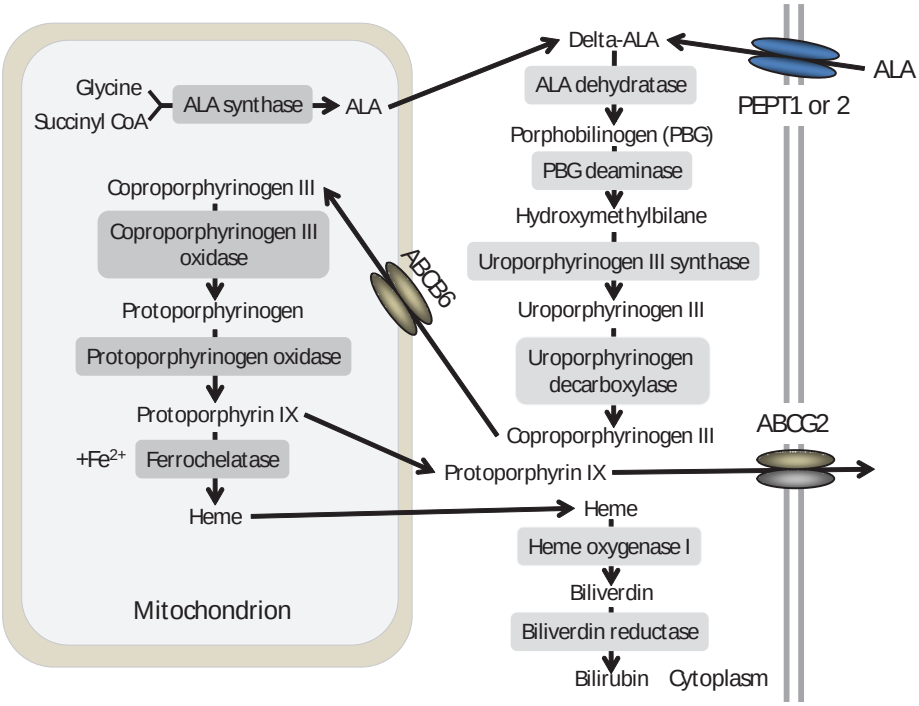

Figure 2. The porphyrin-heme biosynthetic pathway and putative mitochondrial transporters. The first step of heme synthesis occurs in the mitochondrial matrix with the condensation of succinyl CoA and glycine by ALA synthase to generate ALA. Oligopeptide transporters (PEPT1 or PEPT2) are responsible for the import of exogenous ALA from the extracellular space into the cytoplasm of target cells. ALA is converted to coproporphyrinogen III by 4 enzymatic reactions. Then, coproporphyrinogen III is transported back into the mitochondrial intermembrane space (IMS), possibly via ABCB6, where it is converted to protoporphyrinogen by protoporphyrinogen III oxidase. The conversion of protoporphyrinogen to PpIX by protoporphyrinogen oxidase, its transport into the matrix and the addition of Fe^{2+} by ferrochelatase (FECH) to generate heme are coupled processes. The transporter responsible for heme transfer across the outer mitochondrial membrane remains unidentified. Heme formed from porphyrin is catabolized to biliverdin by the microsomal enzyme heme oxygenase 1. Biliverdin is subsequently metabolized to bilirubin by biliverdin reductase. ABCG2 transports porphyrins across the plasma membrane to maintain intracellular porphyrin homeostasis.

Porphyrins are synthesized from glycine and succinyl CoA via a series of enzymatic reactions. The last enzymatic reaction is the conversion of PpIX to heme by FECH, which is located in the inner mitochondrial membrane (Ferreira et al., 1995; Kemmner et al., 2008). Heme formed from porphyrin is catabolized to biliverdin by the microsomal enzyme heme oxygenase 1.

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Subsequently, biliverdin is metabolized to bilirubin by biliverdin reductase. The PEPT1/PEPT2 (influx transporters) can transport exogenous ALA from the extracellular space into the cytoplasm of target cells. ABCB6 is the ABC transporter responsible for the import of coproporphyrinogen III into the mitochondria, whereas ABCG2 transports PpIX across the plasma membrane to maintain intracellular PpIX homeostasis (Takahashi et al., 2011).

Oral-intake of 5-ALA results in the accumulation of PpIX in malignant gliomas. Because of the presence of the blood-brain barrier (BBB), 5-ALA given via the oral route does not usually enter the normal brain tissue. However, it can easily pass through the disrupted BBB found in the glioma tissue (Stummer et al., 2003). PpIX that accumulates in the intracellular compartments of tumor cells exhibits red fluorescence under excitation light of an appropriate wavelength (Takahashi et al., 2011). PpIX-accumulating tumor cells can be visually discerned intraoperatively from the surrounding normal cells that accumulate PpIX to a much lesser extent. To date, little is known about the molecular mechanisms underlying PpIX accumulation in malignant brain tumors after administration of 5-ALA; despite this, in the past decade, studies on ALA focusing on the mechanism of 5-ALA uptake in glioma tissue have been conducted by many research groups. Previously, our group revealed significantly greater down-regulation of FECH expression in glioblastomas than in normal brain tissues and that FECH plays a role in the metabolism of 5-ALA in glioma (Teng et al., 2011). In addition, Takahashi et al. demonstrated that the upregulated expression of the CPOX gene is correlated with the intensity of tumor fluorescence induced by 5-ALA; they also found that the mRNA level of ABCG2 was somewhat lower in the brain tumors with high ALA-induced fluorescence than in those without ALA-induced fluorescence (Takahashi et al., 2011). Therefore, they assumed that both the induction of the CPOX gene and the inhibition of ABCG2 would increase 5-ALA-induced PpIX accumulation and thereby enhance the efficacy of 5-ALA-PDT in malignant brain tumors. In agreement with this hypothesis, Zhao et al. demonstrated that ABCB6 expression levels were greatly elevated in human gliomas than in normal brain tissues and correlated with glioma histologic grade, indicating a crucial role for ABCB6 in ALA metabolism and accumulation of PpIX in gliomas (Zhao et al., 2012).

Further studies are required to explore the detailed mechanism of 5-ALA uptake and improve our knowledge concerning 5-ALA biology in gliomas.

4. 5–ALA guided neurosurgery

Many previous reports describing prognostic factors in patients with malignant gliomas indicate that outcome is associated with the completeness of tumor removal (Stummer et al., 1998a). Improving the prognosis of glioma patients cannot be achieved without considerable effort to remove as much as of the lesion as possible. Since glioma tissue is not easily recognized intraoperatively, methods that easily detect tumor margins during surgery would be extremely beneficial. 5-ALA fluorescence-guided resection is a rapidly growing, novel approach in treating glioma patients to improve the extent of tumor resection with broad applications in both preclinical and clinical settings (Stummer et al., 2000; Stummer et al., 1998b; Stummer et

al., 1998c). In 1998, Stummer et al. first presented a detailed description of the technical principles for 5-ALA fluorescence-guided microsurgical resection of malignant glioma tissue (Stummer et al., 1998a). Subsequently, they performed a prospective study in 52 glioblastoma patients and identified the usefulness of 5-ALA-induced tumor fluorescence for guiding tumor resection. Recent controlled Phase III clinical trials have demonstrated that compared to conventional microsurgery, 5-ALA fluorescence-guided microsurgical resection enables more complete resection of contrast-enhancing lesions and improves progression-free survival in patients with malignant glioma (Pichlmeier et al., 2008; Stummer et al., 2006). After the rapid development of the neurosurgical fluorescence operation microscope, several investigators have recently demonstrated the feasibility of 5-ALA fluorescence-guided resection in patients with glioma (Kuroiwa et al., 1998; Kuroiwa et al., 2001; Sherman et al., 2011). Widhalm et al. also indicated that 5-ALA is a promising marker for intraoperative visualization of anaplastic foci in diffusely infiltrating gliomas without contrast enhancement and showed that the positive predictive value (PVV) of focal 5-ALA fluorescence for World Health Organization (WHO) grade III glioma was 100% (sensitivity 89%) (Widhalm et al., 2010). In addition, Nabavi's group investigated the feasibility and selectivity of 5-ALA-induced fluorescence to guide resection in recurrent gliomas (WHO grade III/IV) and found that 5-ALA fluorescence (weak and strong) had a high PPV (97.2%) in all pathological-appearing tissues obtained from 354 biopsies performed in 36 patients (Nabavi et al., 2009). Based on these findings, they suggested that 5-ALA fluorescence guidance is an effective surgical adjunct in the surgery of recurrent malignant gliomas, and many other trials have displayed similar successful results using 5-ALA fluorescence-guided resection in meningiomas to achieve optimal resection (Coluccia et al., 2010; Kajimoto et al., 2007).

Despite the advantages of 5-ALA fluorescence, successful glioma resection often depends on the neurosurgeon's ability to distinguish residual tumor tissue from surrounding brain tissue even with the assistance of PpIX fluorescence (Teng et al., 2011). The marginal area containing infiltrating glioma cells shows vague fluorescence because the density of the glioma cells in these areas is low and heterogeneous, resulting in insufficient 5-ALA uptake and PpIX accumulation (Utsuki et al., 2006). Conversely, because of the presence of reactive astrocytes and macrophages and leakage of PpIX into the extracellular matrix, false-positive (i.e., the area fluoresced, but no tumor was seen histologically) results in weakly fluorescing normal-appearing tissue are also common (Utsuki et al., 2007). Therefore, more objective and quantitative indicators need to be established for neurosurgeons to improve intraoperative identification of tumor margins. Recently, a quantitative method involving spectroscopic analysis for determining the amount of fluorescence present has been developed, which allows more precise visualization of quantitative fluorescence from tumor tissues (Utsuki et al., 2006; Valdes et al., 2011).

5. 5–ALA mediated-PDT

PDT is a tool for the treatment of certain cancerous and pre-cancerous conditions, especially in the field of dermatology (Buytaert et al., 2007). Among the many kinds of photosensitizers,

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5-ALA has become widely accepted. 5-ALA-PDT is a novel treatment modality for early or superficial cancers, as well as a palliative treatment to a certain extent, that triggers a photodynamic effect similar to that of light in the visible range to produce a rapid PDT response in the targeted tissue. Three types of mechanisms have been identified in the literature as contributing to the rapid PDT response *in vivo*: (1) PDT can directly damage and kill the malignant tumor cells, either by apoptosis or a non-apoptotic mechanism; (2) PDT may produce profound changes in the tumor vasculature, including blood flow stasis, vascular collapse, and/or vascular leakage, that can result in indirect killing of malignant cells; and (3) PDT can promote the release of cytokines and other inflammatory mediators from target cells that induce an inflammatory response and recruit additional host cells to the tumor (MacDonald & Dougherty, 2001; Oleinick et al., 2002). Recent studies have shown that 5-ALA-PDT-induced photodamage causes mitochondrial and nuclear DNA damage. As a result, massive apoptosis occurs owing to mitochondrial release of cytochrome c and activation of caspase-3 and caspase-9 in glioma cells (Inoue et al., 2007; Karmakar et al., 2007). These results indicate that 5-ALA-PDT triggers apoptosis through a mitochondrial pathway.

Compared to the other modalities, 5-ALA-PDT has the advantage of better specificity and lower complication rates, and it is also a good alternative in tumor patients not eligible for surgery. Unfortunately, current 5-ALA-PDT protocols have yet to be widely established in clinical treatment for glioma. This may be partly due to limitations in current PDT regimens and partly due to the therapeutic efficacy of 5-ALA-PDT in preclinical settings (Teng et al., 2011). The reason for 5-ALA-PDT not being standard treatment in malignant glioma may be multifactorial, including the lack of randomized controlled trials and an optimal 5-ALA-PDT regimen. Generally, PDT efficacy depends on parameters such as the photosensitizing agent, irradiance and timing, oxygen, photosensitizer concentration, and different pathologic grade glioma tissue sensitivity to the PDT effect (Teng et al., 2011). Optimizing these parameters is difficult, expensive, and time consuming. The efficacy of 5-ALA-PDT may also be limited by reduced penetration of appropriate light through the target tissue and local acute phototoxicity to normal surrounding brain tissue (Norum et al., 2009). In addition, post-treatment edema and long-lasting skin photosensitivity after PDT of brain tumors are potentially challenging side effects that neurosurgeons have to deal with. Regarding the specificity of PDD for glioma surgery, new techniques allowing more specific accumulation of PpIX in target tumor cells need to be developed. Further studies are required for screening available and specific glioma antigens that are strongly expressed in glioma tissues, but not in normal brain tissues. 5-ALA labeled with antibodies against glioma-specific antigens may be useful in increasing the accumulation of PpIX and the specificity of PDD in glioma tissues. If the specificity and selectivity of 5-ALA-PDT could be improved in glioma tissue, 5-ALA-PDT would certainly be regarded as a promising and competitive alternative in glioma treatment.

6. Perspective

In this review, we focused on the 5-ALA agent as a potential treatment modality for patients with malignant glioma. 5-ALA-induced fluorescence is a useful intraoperative tool for the

visualization of glioma tissue, and 5-ALA-PDT is a promising and alternative adjuvant therapy for photokilling residual neoplastic cells. Although impressive advances in the application of 5-ALA to glioma have been made from many clinical trials, this evolving field still faces important challenges. For example, single imaging in solid malignant glioma tissue with all of these features is not sufficient. Judgment of the extent of resection may still be treacherous within infiltrating parts of brain tumors, where the ratio of background noise of normal brain tissue to signal intensity of PpIX-saturated malignant glioma cells is less pronounced (Hefti et al., 2012; Liao et al., 2012). Recent efforts to boost target-to-background ratios have used a combination of intraoperative 5-ALA-induced fluorescence and 3D MRI imaging or neuronavigation imaging with some success (Liao et al., 2008; Panciani et al., 2012). Furthermore, more objective indicators to measure quantitative PpIX concentrations intraoperatively in the brain tumor margin need to be established. In the future, laboratory and clinical studies should be devoted to 5-ALA-PDT in conjunction with the use of other therapies, which will have maximal effect on the residual tumor after resection. This multiple adjuvant therapy should enhance specificity and allow lowering of the total PDT dose, while still increasing the therapeutic efficacy of PDT. Other proposals should adjust the current PDT treatment regimens, modify the existing photosensitizer, or develop new and more specific photosensitizers. Further studies are needed to elucidate possible mechanisms of 5-ALA uptake that could explain the diversity of intraoperative findings.

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References

[1] Aydin, H, Sillenberg, I, & Von Lieven, H. (2001). Patterns of failure following CT-based 3-D irradiation for malignant glioma. Strahlenther Onkol. 0179-7158, 177(8), 424-431.

[2] Babu, R, & Adamson, C. (2012). Fluorescence-Guided Malignant Glioma Resections. Curr Drug Discov Technol. Vol. No. pp. 1875-6220, 1875-6220.

[3] Blake, E, & Curnow, A. (2010). The hydroxypyridinone iron chelator CP94 can enhance PpIX-induced PDT of cultured human glioma cells. Photochem Photobiol. 1751-1097, 86(5), 1154-1160.

[4] Bottomley, S. S, & Muller-eberhard, U. (1988). Pathophysiology of heme synthesis. Seminars in hematology. 0037-1963, 25(4), 282-302.

[5] Buytaert, E, Dewaele, M, & Agostinis, P. (2007). Molecular effectors of multiple cell death pathways initiated by photodynamic therapy. Biochim Biophys Acta. 0006-3002, 1776(1), 86-107.

[6] Castano, A. P, Mroz, P, & Hamblin, M. R. (2006). Photodynamic therapy and anti-tumour immunity. Nature reviews Cancer. 0147-4175X, 6(7), 535-545.

[7] Coluccia, D, Fandino, J, Fujioka, M, Cordovi, S, Muroi, C, & Landolt, H. (2010). Intraoperative 5-aminolevulinic-acid-induced fluorescence in meningiomas. Acta Neurochir (Wien). 0942-0940, 152(10), 1711-1719.

[8] Hallewin, D, Vanherzeele, M. A, & Baert, H. L. ((1998). Fluorescence detection of flat transitional cell carcinoma after intravesical instillation of aminolevulinic acid. American journal of clinical oncology. 0277-3732, 21(3), 223-225.

[9] Eljamel, M. S, Goodman, C, & Moseley, H. (2008). ALA and Photofrin fluorescence-guided resection and repetitive PDT in glioblastoma multiforme: a single centre Phase III randomised controlled trial. Lasers in medical science. 0268-8921, 23(4), 361-367.

[10] Ferreira, G. C, Franco, R, Lloyd, S. G, Moura, I, Moura, J. J, & Huynh, B. H. (1995). Structure and function of ferrochelatase. J Bioenerg Biomembr. 0014-5479X, 27(2), 221-229.

[11] Gossner, L, Stolte, M, Sroka, R, Rick, K, May, A, Hahn, E. G, & Ell, C. (1998). Photodynamic ablation of high-grade dysplasia and early cancer in Barrett's esophagus by means of 5-aminolevulinic acid. Gastroenterology. 0016-5085, 114(3), 448-455.

[12] Guyon, L, Ascencio, M, Collinet, P, & Mordon, S. (2012). Photodiagnosis and photodynamic therapy of peritoneal metastasis of ovarian cancer. Photodiagnosis Photodyn Ther. 1873-1597, 9(1), 16-31.

[13] Hefti, M, Albert, I, & Luginbuehl, V. (2012). Phenytoin reduces 5-aminolevulinic acid-induced protoporphyrin IX accumulation in malignant glioma cells. J Neurooncol. 1573-7373, 108(3), 443-450.

[14] Iacob, G, & Dinca, E. B. (2009). Current data and strategy in glioblastoma multiforme. J Med Life. 0184-4122X, 2(4), 386-393.

[15] Inoue, H, Kajimoto, Y, Shibata, M. A, Miyoshi, N, Ogawa, N, Miyatake, S, Otsuki, Y, & Kuroiwa, T. (2007). Massive apoptotic cell death of human glioma cells via a mito-

chondrial pathway following 5-aminolevulinic acid-mediated photodynamic therapy. J Neurooncol. 0016-7594X, 83(3), 223-231.

[16] Kajimoto, Y, Kuroiwa, T, Miyatake, S. I, Ichioka, T, Miyashita, M, Tanaka, H, & Tsuji, M. (2007). Use of 5-aminolevulinic acid in fluorescence-guided resection of meningioma with high risk of recurrence- Case report. Journal of Neurosurgery. 0022-3085, 106(6), 1070-1074.

[17] Karmakar, S, Banik, N. L, Patel, S. J, & Ray, S. K. (2007). Aminolevulinic acid-based photodynamic therapy suppressed survival factors and activated proteases for apoptosis in human glioblastoma U87MG cells. Neurosci Lett. 0304-3940, 415(3), 242-247.

[18] Kemmner, W, Wan, K, Ruttinger, S, Ebert, B, Macdonald, R, Klamm, U, & Moesta, K. T. (2008). Silencing of human ferrochelatase causes abundant protoporphyrin-IX accumulation in colon cancer. FASEB J. 1530-6860, 22(2), 500-509.

[19] Kennedy, J. C, & Pottier, R. H. (1992). Endogenous protoporphyrin IX, a clinically useful photosensitizer for photodynamic therapy. J Photochem Photobiol B. 1011-1344, 14(4), 275-292.

[20] Kuroiwa, T, Kajimoto, Y, & Ohta, T. (1998). Development of a fluorescein operative microscope for use during malignant glioma surgery- A technical note and preliminary report. Surgical Neurology. 0090-3019, 50(1), 41-48.

[21] Kuroiwa, T, Kajimoto, Y, & Ohta, T. (2001). Development and clinical application of near-infrared surgical microscope: preliminary report. Minim Invasive Neurosurg. 0946-7211, 44(4), 240-242.

[22] Lefranc, F, Sadeghi, N, Camby, I, Metens, T, Dewitte, O, & Kiss, R. (2006). Present and potential future issues in glioblastoma treatment. Expert Rev Anticancer Ther. 1744-8328, 6(5), 719-732.

[23] Liao, H, Shimaya, K, Wang, K, Maruyama, T, Noguchi, M, Muragaki, Y, Kobayashi, E, Iseki, H, & Sakuma, I. (2008). Combination of intraoperative 5-aminolevulinic acid-induced fluorescence and 3-D MR imaging for guidance of robotic laser ablation for precision neurosurgery. Med Image Comput Comput Assist Interv. No. Pt 2, ISSN:, 11, 373-380.

[24] Liao, H. E, Noguchi, M, Maruyama, T, Muragaki, Y, Kobayashi, E, Iseki, H, & Sakuma, I. (2012). An integrated diagnosis and therapeutic system using intra-operative 5-aminolevulinic-acid-induced fluorescence guided robotic laser ablation for precision neurosurgery. Med Image Anal. 1361-8415, 16(3), 754-766.

[25] Loh, C. S, & Vernon, D. MacRobert, A.J., Bedwell, J., Bown, S.G. & Brown, S.B. ((1993). Endogenous porphyrin distribution induced by 5-aminolaevulinic acid in the tissue layers of the gastrointestinal tract. J Photochem Photobiol B. 1011-1344, 20(1), 47-54.

[26] Loning, M, Diddens, H, Kupker, W, Diedrich, K, & Huttmann, G. (2004). Laparoscopic fluorescence detection of ovarian carcinoma metastases using 5-aminolevulinic acid-induced protoporphyrin IX. Cancer. 0000-8543X, 100(8), 1650-1656.

[27] MacDonaldI.J. & Dougherty, T.J. ((2001). Basic principles of photodynamic therapy. J Porphyr Phthalocya. 1088-4246, 5(2), 105-129.

[28] Nabavi, A, Thurm, H, Zountsas, B, Pietsch, T, Lanfermann, H, Pichlmeier, U, Mehdorn, M, & Glioma, A. L. A. R. (2009). Five-Aminolevulinic Acid for Fluorescence-Guided Resection of Recurrent Malignant Gliomas: A Phase Ii Study. Neurosurgery. 0014-8396X, 65(6), 1070-1076.

[29] Norum, O. J, Selbo, P. K, Weyergang, A, Giercksky, K. E, & Berg, K. (2009). Photochemical internalization (PCI) in cancer therapy: from bench towards bedside medicine. J Photochem Photobiol B. 1873-2682, 96(2), 83-92.

[30] Oleinick, N. L, Morris, R. L, & Belichenko, T. (2002). The role of apoptosis in response to photodynamic therapy: what, where, why, and how. Photoch Photobio Sci. 0147-4905X, 1(1), 1-21.

[31] Panciani, P. P, Fontanella, M, Garbossa, D, Agnoletti, A, Ducati, A, & Lanotte, M. (2012). aminolevulinic acid and neuronavigation in high-grade glioma surgery: results of a combined approach. Neurocirugia. 1130-1473, 23(1), 23-28.

[32] Peng, Q, Warloe, T, Berg, K, Moan, J, Kongshaug, M, Giercksky, K. E, & Nesland, J. M. (1997). Aminolevulinic acid-based photodynamic therapy. Clinical research and future challenges. Cancer. 0000-8543X, 79(12), 2282-2308.

[33] Pichlmeier, U, Bink, A, Schackert, G, & Stummer, W. (2008). Resection and survival in glioblastoma multiforme: an RTOG recursive partitioning analysis of ALA study patients. Neuro Oncol. 1522-8517, 10(6), 1025-1034.

[34] Piotrowski, W. J, Marczak, J, Nawrocka, A, Antczak, A, & Gorski, P. (2004). Inhalations of 5-ALA in photodynamic diagnosis of bronchial cancer. Monaldi Arch Chest Dis. 1122-0643, 61(2), 86-93.

[35] Sherman, J. H, Hoes, K, Marcus, J, Komotar, R. J, Brennan, C. W, & Gutin, P. H. (2011). Neurosurgery for brain tumors: update on recent technical advances. Current neurology and neuroscience reports. 1534-6293, 11(3), 313-319.

[36] Stummer, W, Novotny, A, Stepp, H, Goetz, C, Bise, K, & Reulen, H. J. (2000). Fluorescence-guided resection of glioblastoma multiforme by using 5-aminolevulinic acid-induced porphyrins: a prospective study in 52 consecutive patients. J Neurosurg. 0022-3085, 93(6), 1003-1013.

[37] Stummer, W, Pichlmeier, U, Meinel, T, Wiestler, O. D, Zanella, F, Reulen, H. J, Group, A. L, & Fluorescence-guided, G. S. surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. Lancet Oncol. 1470-2045, 7(5), 392-401.

[38] Stummer, W, Reulen, H. J, Novotny, A, Stepp, H, & Tonn, J. C. (2003). Fluorescence-guided resections of malignant gliomas--an overview. Acta Neurochir Suppl. No. 0065-1419, 88, 9-12.

[39] Stummer, W, Stepp, H, Moller, G, Ehrhardt, A, Leonhard, M, & Reulen, H. J. (1998a). Technical principles for protoporphyrin-IX-fluorescence guided microsurgical resection of malignant glioma tissue. Acta Neurochir (Wien). 0001-6268, 140(10), 995-1000.

[40] Stummer, W, Stocker, S, Novotny, A, Heimann, A, Sauer, O, Kempski, O, Plesnila, N, Wietzorrek, J, & Reulen, H. J. (1998b). In vitro and in vivo porphyrin accumulation by C6 glioma cells after exposure to 5-aminolevulinic acid. J Photochem Photobiol B. 1011-1344, 45(2-3), 160-169.

[41] Stummer, W, Stocker, S, Wagner, S, Stepp, H, Fritsch, C, Goetz, C, Goetz, A. E, Kiefmann, R, & Reulen, H. J. (1998c). Intraoperative detection of malignant gliomas by 5-aminolevulinic acid-induced porphyrin fluorescence. Neurosurgery. discussion 525-526, 0014-8396X, 42(3), 518-525.

[42] Takahashi, K, Ikeda, N, Nonoguchi, N, Kajimoto, Y, Miyatake, S, Hagiya, Y, Ogura, S, Nakagawa, H, Ishikawa, T, & Kuroiwa, T. (2011). Enhanced expression of coproporphyrinogen oxidase in malignant brain tumors: CPOX expression and 5-ALA-induced fluorescence. Neuro Oncol. 1523-5866, 13(11), 1234-1243.

[43] Teng, L, Nakada, M, Zhao, S. G, Endo, Y, Furuyama, N, Nambu, E, Pyko, I. V, Hayashi, Y, & Hamada, J. I. (2011). Silencing of ferrochelatase enhances 5-aminolevulinic acid-based fluorescence and photodynamic therapy efficacy. Br J Cancer. 1532-1827, 104(5), 798-807.

[44] Toda, M. (2008). Intraoperative navigation and fluorescence imagings in malignant glioma surgery. The Keio journal of medicine. 1880-1293, 57(3), 155-161.

[45] Utsuki, S, Oka, H, Sato, S, Shimizu, S, Suzuki, S, Tanizaki, Y, Kondo, K, Miyajima, Y, & Fujii, K. (2007). Histological examination of false positive tissue resection using 5-aminolevulinic acid-induced fluorescence guidance. Neurol Med Chir (Tokyo). 0470-8105, 47(5), 210-213.

[46] Utsuki, S, Oka, H, Sato, S, Suzuki, S, Shimizu, S, Tanaka, S, & Fujii, K. (2006). Possibility of using laser spectroscopy for the intraoperative detection of nonfluorescing brain tumors and the boundaries of brain tumor infiltrates. Technical note. J Neurosurg. 0022-3085, 104(4), 618-620.

[47] Valdes, P. A, Leblond, F, Kim, A, Harris, B. T, Wilson, B. C, Fan, X. Y, Tosteson, T. D, Hartov, A, Ji, S. B, Erkmen, K, Simmons, N. E, Paulsen, K. D, & Roberts, D. W. (2011). Quantitative fluorescence in intracranial tumor: implications for ALA-induced PpIX as an intraoperative biomarker. Journal of Neurosurgery. 0022-3085, 115(1), 11-17.

[48] Wallner, K. E, Galicich, J. H, Krol, G, Arbit, E, & Malkin, M. G. (1989). Patterns of failure following treatment for glioblastoma multiforme and anaplastic astrocytoma. Int J Radiat Oncol Biol Phys. 0360-3016, 16(6), 1405-1409.

[49] Widhalm, G, Wolfsberger, S, Minchev, G, Woehrer, A, Krssak, M, Czech, T, Prayer, D, Asenbaum, S, Hainfellner, J. A, & Knosp, E. (2010). Aminolevulinic Acid Is a Promising Marker for Detection of Anaplastic Foci in Diffusely Infiltrating Gliomas With Nonsignificant Contrast Enhancement. Cancer. 0000-8543X, 116(6), 1545-1552.

[50] Zhao, S. G, Chen, X. F, Wang, L. G, Yang, G, Han, D. Y, Teng, L, Yang, M. C, Wang, D. Y, Shi, C, Liu, Y. H, Zheng, B. J, Shi, C. B, Gao, X, & Rainov, N. G. (2012). Increased Expression of ABCB6 Enhances Protoporphyrin IX Accumulation and Photodynamic Effect in Human Glioma. Annals of surgical oncology. Vol. No. pp. 1534-4681, 1534-4681.