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Breast Cancer and Current Therapeutic Approaches: From Radiation to Photodynamic Therapy

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1. Introduction

Breast cancer is one of the oldest known forms of cancer in humans and it has been mentioned in almost every period of human history. Since the time of the ancient Egyptians and Greeks, there has been no cure but only treatment for this disease. In the 18th century, different theories about the origin of breast cancer were developed. During this period, an important link between breast cancer and the lymph nodes was established. The assumption that cancer was a localized disease led to the rise of the surgical approach in breast cancer treatment. Since the work of William Halstead (1882), radical mastectomy (removal of breast tissue, lymph nodes and chest tissue) remained the standard for almost 100 years (Leopold, 1999; Olson, 2002). With the advance in science, novel therapeutic and diagnostic opportunities came into use in breast cancer treatment. Introduction of radiation at the beginning of the 20th century enabled tumour size to be reduced before surgery. Another major breakthrough came with the use of chemotherapy in the 1940s. Their combination with surgery offers another powerful treatment modality. The discovery by Beatson in 1895 that removal of the ovaries results (in some cases) in reduction of breast tumours led to the later elucidation of oestrogen`s role in breast cancer growth (Forrest, 1982). Research in pharmaceutical approaches to breast cancer/oestrogen management ended in the development of aromatase inhibitors (AIs) and selective oestrogen receptor modifiers. An important step came in 1998, when the US Food and Drug Administration (FDA) approved trastuzumab for the treatment of HER2 positive metastatic breast cancer. Treatment with trastuzumab has a major impact on the survival of a subset of patients with resistant and hard to treat breast tumours (Shepard et al., 2008). With the introduction of mammography, early detection of breast cancer was made possible. Mammography screening combined with more precise therapy was shown to reduce breast cancer mortality between 24.9 and 38.3% (Berry et al., 2005). Several other detection methods including magnetic resonance, ultrasound and 3D digital mammography have been developed and are now used in the fight against breast cancer (Gilbert, 2008; Hellerhoff, 2010).

2. Current therapeutic approaches

2.1 Radiotherapy

Radiation therapy uses high-energy x-rays to destroy cancer cells. This therapy usually follows lumpectomy to eliminate any microscopic cancer cells in the remaining breast tissue.

Sometimes radiation therapy is also given after a mastectomy, but only if there is a high risk of cancer recurring in that area. Early studies on the use of adjuvant radiotherapy are difficult to interpret owing to poor radiotherapy techniques, inappropriate dosage or a variety of confounding variables within a particular trial. The results of clinical studies have confirmed that adjuvant radiotherapy will reduce the risk of local recurrence and produce a reduction in breast cancer deaths for tumours of <5 cm with involved nodes (Fernando, 2000).

Furthermore, adjuvant radiotherapy combined with tamoxifen has been shown to produce an improvement in both local control and survival in postmenopausal node-positive patients who have undergone mastectomy. Adjuvant radiation combined with systemic chemotherapy has a significant effect on local recurrence and probably on survival in node-positive patients after mastectomy (Fernando, 2000).

Radiotherapy has undergone significant technological advances during the last 20 years, although its use in breast cancer was relatively limited until recently. The major recent changes in the use of radiotherapy for breast cancer have been the following: the establishment of partial breast irradiation as an option for therapy in early stage disease; the revival of hypofractionated therapies for breast-only therapy; the clearer definition of the role of post-mastectomy irradiation; and the continuing investigation as to which patients having conservative surgery do not need radiation therapy (Powell, 2010). Nowadays, Memorial Sloan-Kettering Cancer Center (New York, NY, USA) offers several newer forms of radiation therapy for breast cancer, which include intensity-modulated radiation therapy, radiation delivered in the prone position and image-guided radiation therapy.

In addition to cytocidal effects, ionizing radiation has been shown to cause a plethora of changes on both the cancer cells and tumour stroma, critical in determining its therapeutic success (Formenti & Demaria, 2008). Many of these changes have been proven in experimental systems to affect the ability of the immune system to reject the tumour (Demaria & Formenti, 2007). In this regard, radiation-induced upregulation of Fas/CD95 (Chakraborty et al., 2003) and MHC 1 (Reits et al., 2006) on cancer cells and VCAM 1 (Lugade et al., 2008) on tumour-associated endothelia must be considered. Moreover, Matsumura et al. (2008) showed that radiation enhances the release of chemokine CXCL16 by human and mouse breast cancer cells, which is very important for efficient recruitment of antitumour T cells and tumour inhibition following treatment with radiation and CTL-associated antigen 4 blockade.

Recently, targeted intraoperative radiotherapy impaired the stimulation of breast cancer cell proliferation and invasion caused by surgical wounding. Indeed, the fluid from wound drainage stimulated proliferation, migration and invasion of breast cancer cells. The observed effect was negated when wound drainage fluid was obtained from patients who had undergone intraoperative radiotherapy (Belletti et al., 2008). More clinical studies are needed to support the hypothesis that immune mechanisms underlie the effect of local control on systemic outcome (Formenti & Demaria, 2008).

2.2 Chemotherapy

Many specialists recommend chemotherapy following surgery to kill cancer cells that may have spread outside the breast (adjuvant therapy). Chemotherapy might be recommended before surgery (neoadjuvant therapy) if the breast tumour is large, the lymph nodes are involved or the tumour is attached to the chest wall muscles, and also in the cases of inflammatory breast cancer.

Anthracyclines were considered the gold standard of adjuvant chemotherapy until the late 1990s. However, long-term treatment with side effects such as cardiac toxicity and leukemia/myelodysplastic syndrome can negate their benefits. The real benefit from anthracyclines could be felt by patients with topoisomerase II α amplification, which is usually associated with HER2 amplification. Overall, the anthracycline regimens (for example 5-fluorouracil, doxorubicin and cyclophosphamide – FAC; 5-fluorouracil, epirubicin and cyclophosphamide – FEC, or doxorubicin and cyclophosphamide – AC) are associated with reduction in the risk of recurrence by 11.2% and in the risk of death by 16 %, compared with combinations including cyclophosphamide, methotrexate and 5-fluorouracil (Lopez-Tarruella & Martin, 2009).

Although the precise role of taxanes is uncertain, based upon the data from first-generation taxane trials it is reasonable to consider taxane therapy in women with an elevated risk of relapse where endocrine sensitivity is absent or incomplete (Bedard & Cardoso, 2008). As the number of treatment options increases, the need to define a set of criteria to select those patients who will benefit from each treatment regimen or strategy becomes a priority (Lopez-Tarruella & Martin, 2009).

About three quarters of breast cancer cells express oestrogen and/or progesterone receptors, therefore the first targeted breast cancer therapy was the antioestrogen one. The first such therapy approved for the treatment of breast tumours was the therapy involving tamoxifen. Although first studies showed positive effects of tamoxifen, adverse effects causing endometrial cancer and thromboembolism were later shown by Fisher et al. (1994) and Jordan (1995).

Because of higher production of oestrogens in breast cancer tissues in comparison to noncancerous ones, another very attractive target for breast cancer treatment is aromatase (Harada, 1997). Multiple clinical studies have demostrated the efficacy and reduced side effects of AIs vs. tamoxifen. However, their benefit is limited by the resistance induced through the crosstalk between oestrogen receptor and other signalling pathways, particularly MAPK and PI3K/Akt. Interfering with these other signalling pathways is an attractive strategy to circumvent the resistance to AIs in breast cancer. Several clinical trials are under way to evaluate the role of these novel target therapies to reverse resistance to AIs. These agents include MEK inhibitors, Raf inhibitors, PI3K inhibitors, mTOR inhibitors and Akt inhibitors (Chumsri et al., 2011).

Fulvestrant (selective oestrogen receptor downregulator) is recommended for second-line therapy after failure of tamoxifen, and for third-line therapy after failure of tamoxifen and AIs. Other third-line agents used after the failure of other options include progestins, androgens or high-dose oestrogens (Beslija et al., 2007).

Several multigene markers that predict relapse more accurately than classical clinicopathologic features have been developed. The 21-gene assay was developed specifically for patients with oestrogen receptor ER-positive breast cancer, and has been shown to predict distant recurrence more accurately that classical clinicopathologic features in patients with ER-positive breast cancer and negative axillary nodes treated with adjuvant tamoxifen (Sparano & Paik, 2008). Another 70-gene profile is a new prognostic tool that has the potential to greatly improve risk assessment and treatment decision-making for early breast cancer. Its prospective validation is currently under way through the MINDACT (Microarray in Node-Negative Disease May Avoid Chemotherapy), a 6000-patient randomized, multicentric trial (Cardoso et al., 2008).

2.3 Therapy of HER2 positive breast cancers

The HER2 oncoprotein is an important therapeutic target in the treatment of invasive breast cancers associated with poor disease-free survival and resistance to chemotherapy (Nahta et al., 2006). HER2 status is a significant prognostic factor for local-regional disease progression. Patients with positive HER2 status had a local-regional disease progression-free rate of 59% compared with 92% for patients with negative HER2 status (Haffty et al., 2004).

Although the application of monoclonal antibody against HER2 – trastuzumab showed beneficial effect when combined with docetaxel and platinum salts (Pegram et al., 2004) or paclitaxel and carboplatin (Perez et al., 2005; Robert et al., 2006), its use beyond first-line therapy might develop resistance to this agent. In this regard, inhibition of PTEN (Nagata et al., 2004), overexpression of IGF-IR (Lu et al., 2001) and MUC4 (Nagy et al., 2005) and increased level of VEGF protein (du Manoir et al., 2006) could play a significant role. In order to make trastuzumab treatment more effective after disease progression, new agents targeting the HER2 pathway have been developed. The number of HER-targeting agents include antibody, small tyrosine kinase inhibitor (TKI) molecules, mTOR inhibitors, Hsp90 inhibitor, farnesyltransferase inhibitor and PI3K inhibitor (Morrow et al., 2009). One of the TKI small molecules, lapatinib, has been approved (in combination with capecitabine) by the FDA in the treatment of patients with advanced or metastatic HER2 positive breast cancer which progresses after trastuzumab, anthracyclines and taxanes (Morrow et al., 2009).

2.4 Therapy of HER2 negative breast cancers

Trastuzumab has improved outcomes in breast cancer patients with HER2 overexpressing tumours. However, systemic treatment for patients with HER2 negative diseases is still limited to endocrine and cytotoxic therapies. Anthracyclines and taxanes used in early-stage disease reduce the available therapeutic options for patients with relapsed disease. Treatment choices are limited in patients with triple-negative breast cancer (do not express HER2 and hormone receptors), where the prognosis is usually poor (Miles, 2009). These tumours are sensitive to platinum compounds and their DNA damaging effect, because of downregulation of BRCA-1 (DNA repair protein) (James et al., 2007). The results of combined platinum and taxane (docetaxel) therapy in patients with triple-negative metastatic breast cancer ongoing from phase III trial are expected in 2012 (Miles, 2009).

There are some novel chemotherapeutic agents in clinical development. One of them, nab-paclitaxel (nanoparticle albumin-bound paclitaxel), has been approved for metastatic breast cancer patients with failed first-line therapy. The second very interesting group of agents consists of microtubule stabilizing anticancer drugs, epothilones (ixabepilone has been approved only in the USA), which are desirable for patients with anthracycline and taxane resistant tumours (Thomas et al., 2007). Another special group of drugs comprises anti-angiogenic agents which target and inhibit VEGF (bevacizumab) (Miller et al., 2007) or VEGF receptor, as well as other receptor tyrosine kinases (e.g. sunitinib, pazopanib, axitinib, sorafenib) (Miller et al., 2005). Other very attractive candidates for single or combined therapy of patients with metastatic breast cancer are also EGFR inhibitors (von Minckwitz et al., 2005), mTOR inhibitors (Chan et al., 2005), Ras cascade inhibitors (Normanno et al., 2005) and PARP inhibitors (Bryant et al., 2005; Nguewa et al., 2006).

Nowadays, targeted therapy with anti-sense nucleotides, inhibitors of apoptosis proteins, proteasome system inhibitors as well as cyclin-dependent kinase inhibitors are in phase I-III of clinical studies (Schlotter et al., 2008). One of the greatest challenges in breast cancer

treatment is the delivery of miRNA inhibitors or miRNA mimics specifically to tumour cells, which will probably become reality in the near future (O'Day & Lal, 2010).

2.5 Therapeutic potential of natural compound genistein

Genistein (GE) belongs in the isoflavone class of flavonoids, with soy beans as a major source (Akiyama et al., 1987). The flavonoids display a wide spectrum of pharmacological activities, but their anticancer activity is the most important (Lee et al., 2002). In particular, GE has proven to be a valuable tool for the inhibition of cancer metastasis, exerting effects on both the initial step of primary tumour growth as well as the later steps of the metastatic cascade. This isoflavonoid inhibits cell growth and induces cell death in numerous types of cancer cells (Yeh et al., 2007). Data obtained to date suggest that the anticancer effects of GE result from various mechanisms, including the regulation of cell cycle progression (Constantinou et al., 1998), inhibition of tyrosine kinases (Akiyama et al., 1987) and inhibition of matrix metalloproteinase (Xu & Bergan, 2006).

A number of studies have suggested that GE may induce apoptosis in several breast cancer cell lines and produce synergistic inhibitory effects when combined with cancer therapies. GE has been shown to induce apoptosis in the high invasive MDA-MB-231 and low invasive MCF-7 breast cancer cell lines at relatively high concentrations of 10 – 100 μM (Li et al., 2008; Nomoto et al., 2002). The concentration as well as the cell type are critical determinants of the isoflavone effect (Pavese et al., 2010). In accord with this fact, GE has been shown to have biphasic proliferative effect in breast cancer cells, inhibiting *in vitro* cell proliferation at high concentrations (>10 μmol/l), while stimulating proliferation of oestrogen receptor positive cells (but not oestrogen receptor negative cells) at lower concentrations (<10 μmol/l) (Zava & Duwe, 1997). A number of studies have shown that GE at higher concentrations affects multiple intracellular targets and has impact on tumour cells independently of the oestrogen receptors (Constantinou et al., 1998), but as a phytooestrogen, GE can bind to both oestrogen receptors (ERα/ERβ), though it has a higher affinity for ERβ than ERα (Muthyala et al., 2004). Concerning the role of oestrogen receptors, Liu et al. (2002) demonstrated that both RT-PCR and immunohistochemical staining showed significantly higher ERα expression in cancerous human breast than in normal breast, while ERβ was higher in normal human breast than in cancerous breast. On the other hand, up-regulation of ERβ in breast cancer cells by trichostatin A, a histone deacetylase inhibitor, led to induced sensitivity to tamoxifen (Jang et al., 2004). GE could therefore be used as a potential chemotherapeutic agent against breast cancer of the ERα-negative and ERβ-positive type (Rajah et al., 2009).

The study undertaken by Xu et al. (2009) was the first to demonstrate the inhibition of prometastatic processes in humans through therapeutic application of GE, even with low blood concentration of GE (approximately 140 nM).

According to studies concerned with GE's weak oestrogenic activity (Messina et al., 2006), it seems that the effect of GE on breast cancer depends on the nature of the oestrogenic environment in which the study is conducted. In this regard, if endogenous oestrogen is low, GE can bind the ER receptor and exert progrowth effects upon responsive systems. On the other hand, if oestrogen is high and potent, GE can act as a competitor to oestrogen and thus antagonize this hormone´s effect. In addition, gene expression levels of BRCA-1 and BRCA-2, breast tumour suppressor genes, were maintained over the 3-year period in the group administered with GE, whereas the placebo group showed decreased levels of both BRCA-1 and BRCA-2 gene products (Marini et al., 2008). The recent nested case control

study by the Japan Public Health Center shows statistically significant inverse association between GE and the risk of breast cancer over a 10-year period. Furthermore data from this study suggest that even at the relatively low concentrations achievable from dietary intake alone (highest plasma level 353.9 ng/ml), GE poses a risk-reducing rather than a risk-enhancing effect on breast cancer (Iwasaki et al., 2008). Similarly, a prospective study in the Dutch population examined the association between plasma levels of isoflavones (daidzein, GE, glycitein, O-desmethylangolensin and equol) or lignans (enterodiol and enterolactone) and breast cancer risk. The result of the study was that high circulating GE levels are associated with reduced breast cancer risk (Verheus et al., 2007). In contrast, a prospective study of European women found no protective effect of high levels of GE and other phytooestrogens (in the blood and urine) against breast cancer (Ward et al., 2008).

3. Photodynamic therapy (PDT)

There is a plethora of approaches to cancer therapy that may be sorted into various categories in many different ways. But generally speaking, there are treatments based on biologically relevant actions of chemical compounds or physical effects. Irradiation, either in the form of electromagnetic waves or accelerated particles, has earned its stable position in the oncological armoury, and γ-irradiation has been successfully used for decades. However electromagnetic radiation with longer wavelengths and lower energy is also used in modern medicine for various intents. Direct exposure to non-ionizing radiation for therapeutic use (natural light, UVB or UVA radiation) known as a phototherapy is usually applied for treatment of skin conditions such as dermatitis, psoriasis or vitiligo. However, it has also found its place in other medical areas, with particular applications in psychiatry in the treatment of internal depressions, sleeping changes, or the circadian rhythm (Ledo & Ledo, 2000).

The physical and chemical "approaches" may also be combined together to ensure higher therapeutic efficiency, or work together for diagnostic purpose. Combination of a photosensitizing chemical substance followed by electromagnetic non-ionizing radiation is known as a photochemotherapy, and is typically administered using psoralen (as the photosensitizer) and long-wavelength ultraviolet radiation (UVA). Furthermore, photosensitizer and light may also be combined with oxygen to get a highly effective therapeutic paradigm named as "photodynamic" therapy (PDT). This might be subsumed into the photochemotherapy subset, and together they belong in the phototherapy family. Photodynamic therapy employs visible light, often in the red or near IR part of the spectrum. The energy of photons absorbed by the photosensitizer is generally used for transformation of oxygen into highly-reactive intermediate oxygen radicals. The main advantage of this approach is the combination of three inoffensive entities which together create a highly toxic conjunction, and so it has also found applications in the treatment of a wide range of malignancies (Ledo & Ledo, 2000).

By its nature, PDT is a flexible and versatile therapeutic approach depending on the nature of the photosensitive compound, its concentration and incubation time, on the wavelength of light radiation, fluence rate and light dose, the time between drug administration and its activation (Kuliková et al., 2010), as well as on the histological origin of tissue and the oxygen pressure in it (Agostinis et al., 2002). All these factors modulate three independent processes contributing to tumour destruction by PDT: direct cell death, destruction of tumour vasculature causing tumour ischemia, and activation of an immune response

(Buytaert et al., 2007). Practical application of PDT is straightforward and based on three elementary steps: administration of a photosensitive compound, its selective accumulation in neoplastic tissue, and irradiation of the tissue with visible light of an appropriate wavelength (Oleinick et al., 2002). Depending on the part of a body being treated, the photosensitizing agent may be either injected into the bloodstream or applied locally to the skin. After the drug is absorbed by the cancer cells, a light source is applied only to the area to be treated. *In vivo* studies have shown that the PDT can work as well as surgery or radiation therapy, but unlike both of them PDT can also alert the immune system and stimulate specific immune responses for treatment of malignant as well as non-malignant diseases (Qiang et al., 2008). Topical PDT is well tolerated and leads to excellent aesthetic results with only minor side effects (Fritsch & Ruzicka, 2006), so it is an excellent choice for non-malignant applications such as psoriasis, viral-induced diseases or acne vulgaris. As the photosensitizers are also fluorescent, they are applicable as a highly efficient contrasting method in detection of tumours *via* so-called "photodynamic diagnostics" (PDD). Hypericin for example has proved to be very effective in fluorescence cystoscopy of bladder cancer (Jichlinski & Leisinger, 2005).

Until recently, all the advantages of PDT were believed to be compromised by the weak penetration of visible light into body tissues. It was therefore considered effective only for treatment of superficial cancers (Hopper, 1996) located on or just under the skin or in the lining of internal organs. Although the limited penetration issue cannot be eliminated, the use of specially-designed catheters and fibre optics can distribute light in three-dimensional space. Simultaneous irradiation with a set of catheters accurately combined in space can efficiently irradiate a large tumour mass. Moreover, using fibre optics, visible light is much easier to distribute in comparison to high-energy particle or γ-radiation. Moreover, the lower penetration of visible light may even be advantageous in the case of anatomically complex tumours, and may help to protect sensitive histological structures. Slow body clearance and therefore long-lasting skin sensitivity to light is another drawback of PDT that may be managed with special precautions (Reddy et al., 2006). Furthermore, new prospects have been introduced lately thanks to experiments with different delivery systems such as nanoparticles (Simon et al., 2010).

Since PDT may be targeted precisely, it is in many cases less invasive than surgery, and unlike radiation it can be repeated several times at the same site, if necessary. Thanks to these attributes, PDT does not demonstrate any long-term side effects when used properly.

3.1 Mechanism of PDT

The molecular mechanism of PDT is based on absorption of photons, which transforms the photosensitizer from the ground singlet into the excited state. Release of accumulated energy and consequent relaxation of the molecules back to the ground state might be accomplished either by emitting fluorescence that can be used by PDD for diagnostic purposes (Berg et al., 2005) or by intersystem crossing to a relatively stable (in range μs – ms) excited triplet state followed by generation of radicals (Takemura et al., 1989).

Relaxation from the triplet state can generate either free radicals or radical ions by hydrogen atom extraction or electron transfer to biological substrates (such as membrane lipids), solvent molecules or oxygen (Berg et al., 2005). The radicals generated by the photosensitizer can interact with ground-state molecular oxygen to produce superoxide anion (O^{2-}) radicals, hydrogen peroxides (H_2O_2) and hydroxyl radicals (·OH) (so-called "Type I reaction"). Direct transfer of energy from the triplet state photosensitizer to the

ground state molecular oxygen forms non-radical but highly reactive singlet oxygen ($^1O_2$) (so-called "Type II reaction"), which is of higher significance for PDT action (Niedre et al., 2002). On the other hand, production of superoxide anions in the Type I reaction can form hydrogen peroxide able to diffuse through the membranes, so it might be toxic for neighbouring cells. Addition of another electron can lead to generation of two hydroxyl radicals, the most dangerous member of the reactive oxygen species (ROS) family with ability to attack and oxidize any compound of biological origin (Plaetzer et al., 2005). Both oxygen-dependent reactions occur simultaneously, but the ratio between them depends on the photosensitizer and available substrate molecules (Berg et al., 2005). Oxidative damage in the cell induced by ROS generated *via* PDT also depends on the intracellular localization, affects different cell organelles and induces cell death (Ahmad & Mukhtar, 2000).

Since the photogenerated singlet oxygen has a very short life and very limited diffusion in biological systems (half-life: <0.04 μs, radius of action: <0.02 μm), the primary molecular targets of the photodynamic process have to reside within a few nanometers from the dye (Moan & Berg, 1991). Therefore it is generally accepted that subcellular localization of the photosensitizer coincides with the primary site of photodamage. The plasma membrane, mitochondria, lysosomes, Golgi apparatus and endoplasmic reticulum (ER) are the most frequent targets of PDT. Moreover, since most dyes do not accumulate in cell nuclei, PDT has generally much lower potential of causing DNA damage, mutations and carcinogenesis as compared to that induced by X-radiation at equitoxic fluencies/doses (Oleinick et al., 2002). Even so, some studies have reported the relocalization of certain photosensitizers after irradiation (Berg et al., 1991; Marchal et al., 2007), suggesting that besides the primary site, photodamage can be rapidly propagated to other subcellular locations. Since photo-generation of singlet oxygen and radicals is limited to the light period when the photosensitizer is activated, the fluency rate of the light source and therefore the time frame of the administration might also affect the PDT efficiency, as the photosensitizer moving during light administration may generate different damage patterns.

Disregarding this issue, photosensitizers localized in the mitochondria and ER tend to promote apoptosis, while those targeting the plasma membrane or lysosomes can either delay or even block apoptosis and thereby also any arising predisposition for necrosis (Kessel et al., 1997). Necrosis (apart from massive cellular destruction leading to bioenergetic catastrophe) may under given circumstances be considered, regarding the concept of programmed necrosis (Proskuryakov et al., 2003), as a form of programmed cell death. Similarly the autophagic repair process may transform into a programmed event, possibly executable after irreparable photodamage to crucial cellular structures (Buytaert et al., 2006b; Buytaert et al., 2006a). Photoactivated photosensitizers with a prevalent mitochondrial localization, (e.g. porphyrogenic sensitizers and phthalocyanine-related compounds) rapidly mediate $\Delta\Psi m$ dissipation accompanied by cytochrome *c* release and a drop in intracellular ATP levels (Almeida et al., 2004; Oleinick et al., 2002). However, the mitochondria are also critical executers of lethal pathways emanating from photodamage to other subcellular sites or organelles, although in this case the release of apoptogenic proteins from the mitochondria is delayed (Buytaert et al., 2007). On the other hand, in some cases (e.g. hypericin) PDT may trigger ER Ca^{2+} store emptying as a consequence of sarco(endo)plasmic-reticulum Ca^{2+}-ATPase (SERCA2) protein level loss, initiated by ER-associating hypericin (HY) irradiation (Buytaert et al., 2006a). Intracellular Ca^{2+} overload, with consequent mitochondrial Ca^{2+}-uptake, increased cellular pro-oxidant state and the

generation of free fatty acids, such as those produced by phospholipase A2, are known factors favouring permeability transition pore (PTP) opening (Rasola & Bernardi, 2007).
Nevertheless, necrosis as a type of programmed cell death is not a result of one well-described signalling cascade but is the consequence of extensive crosstalk between several biochemical and molecular events at different cellular levels. It seems that serine/threonine kinase RIP1 (receptor interacting protein), which contains a death domain, may act as a central initiator. Fluctuations in calcium level and ROS accumulation may directly or indirectly provoke damage to proteins, lipids and DNA, culminating in disruption of organelle and cell integrity (Festjens et al., 2006).
It has been shown as well that PDT may induce non-apoptotic cell death associated with the induction of autophagy (Buytaert et al., 2006b). Due to the high reactivity of photogenerated ROS, it is not surprising that autophagy is initiated in an attempt to remove heavily-damaged organelles or to degrade large aggregates of cross-linked proteins produced by photochemical reactions, which cannot be removed by the ubiquitin–proteasome system or by the degradation associated with ER. Since autophagy is a self-limiting process, it is possible that its persistence results in metabolic and bioenergetic collapse, which is causative for cell death (Buytaert et al., 2006b).
It is evident that the type of ROS and site of their production within the cell represents the vital death switch mechanism which regulates transition among cell death types. However, apoptosis is a highly-regulated event and there are often various changes in cell signalling pathways which are present primarily in the cell or evoked by PDT itself. For example increased expression of anti-apoptotic proteins from the Bcl-2 family, often found in about half of the various human cancers (Reed, 1998), could impose a certain resistance to apoptosis and switch the balance towards necrosis in some cell types (Agostinis et al., 2002). Likewise, we have documented that p53-deficient cells, although similarly sensitive to PDT with HY as their wild-type p53-expressing opposites, tend to die by necrosis (Mikeš et al., 2009).

3.2 Hypericin

Hypericin, a naturally-occurring photosensitive compound, is a naphthodianthrone derivative synthesized by the plant St. John's Wort. Among others it possesses properties suitable for PDT (Čavarga et al., 2005; Chan et al., 2009) and PDD (Thong et al., 2009). Peculiar attributes of this photosensitizer are high efficiency in production of singlet oxygen (Redmond & Gamlin, 1999) and superoxide anions after irradiation with light wavelength around 600 nm and low or no toxicity in the dark (Jacobson et al., 2001; Miadoková et al., 2009). Photoactivated HY is known to induce changes at cellular as well as vascular level or even affect CD8+ T cell-mediated cytotoxicity (Lavie et al., 2000). At the cellular level, activated HY induces many events, more or less specific, such as membrane lipid peroxidation (Chaloupka et al., 1999), increased activity of superoxide dismutase, decreased glutathione concentration (Hadjur et al., 1996) or injury to the mitochondria (Vantieghem et al., 2001). One relatively specific example seems to be its ability to inhibit various enzymes. HY, whether light-activated or not, has been found to inhibit an extensive spectrum of Ser/Thr protein kinases (Blank et al., 2001), protein tyrosine kinases or even HIV-1 reverse transcriptase (Schinazi et al., 1990), and it also seems to play a role in the onset of multidrug resistance phenotype (Jendželovský et al., 2009). Its fluorescence is applicable in the detection of tumours *via* PDD and has proved to be very effective in fluorescence cystoscopy of bladder cancer (Jichlinski & Leisinger, 2005).

The cytotoxic effects of HY are generally considered to be oxygen- and light-dependent (Huygens et al., 2005), as the absolute elimination of HY photocytotoxicity in a hypoxic environment (Delaey et al., 2000) together with the absence of effect on mitochondrial function have been documented (Utsumi et al., 1995). On the other hand, light-independent inhibition of some enzymes (Johnson & Pardini, 1998) as well as anti-metastatic and cytotoxic activity of HY in the dark have been demonstrated both *in vitro* (Blank et al., 2001) and *in vivo* (Blank et al., 2004). However, the light-independent action of HY generally requires markedly higher doses. The significance of proper light regime has also been suggested by us (Kuliková et al., 2010; Sačková et al., 2005) and it is now beyond doubt that low light doses induce photo-tolerance. Discontinuity time proved to be crucial.

The mode of cell death may be significantly governed by HY uptake and intracellular localization, too. It is mostly reported as localizing in the endoplasmic reticulum and/or Golgi apparatus, as well as in the lysosomes and mitochondria (Agostinis et al., 2002; Kaščáková et al., 2008). For this reason, rapid loss of $\Delta\Psi_m$, subsequent cytochrome *c* release, caspase-3 activation and apoptosis all occur as a result of PDT with hypericin (HY-PDT). Since the photocytotoxic action of HY represents a massive impact on various cellular targets, cytochrome *c* release as well as caspase-3 activation and apoptosis can be suppressed, for example in cells over-expressing Bcl-2, but not ΔΨm loss (Hadjur et al., 1996; Vantieghem et al., 2001).

Although cells sensitized by activated HY show all of the elementary signs of apoptosis, recent studies have revealed that cell death may proceed *via* both caspase-dependent or -independent pathways. Initial experiments linked HY-induced apoptosis with inhibition of protein kinase C (PKC) (Couldwell et al., 1994); however, inhibition of PKC was later proven to be insufficient to cause apoptosis (Weller et al., 1997). On the other hand, HY also activates rescuing responses, chiefly governed by activation of p38MAPK (Hendrickx et al., 2003) and the genes that are under its control (Buytaert et al., 2008; Chan et al., 2009).

Hypericin's cytotoxicity or photocytotoxicity may also be a result of its interaction with expression and/or activity of some specific enzymes. Some of them, like PI3K, PKC, protein tyrosine kinase activities (PTK) of the epidermal growth factor receptor (EGF-R) and the insulin receptor are closely related to tumourigenesis, survival or proliferation regulation. The Ser/Thr protein kinases (e.g. protein kinase CK-2 or mitogen-activated kinase) are also extremely sensitive to inhibition even in nanomolar concentrations, and have also proved to be irreversible after irradiation (Agostinis et al., 1995). As the light-dependent action of HY is based on induction of oxidative stress, the action of antioxidant enzymes has been tested *in vivo*. The inhibition of glutathione reductase was highly effective even in the nanomolar range of HY, whether light-activated or not (Johnson & Pardini, 1998). The inhibition of selenium-dependent glutathione peroxidase, glutathione S-transferase and superoxide dismutase proved to be efficient in micromolar concentrations and light-dependent.

Evaluation of the inhibitory effect of St. John's Wort towards human cytochromes P450 (CYP) has revealed possible interactions of its constituents. HY *per se* proved to be a competitive inhibitor of CYP2C9, CYP2D6 and CYP3A4 with IC50 below 10 μM (Obach, 2000). Besides CYP3A4, the inhibition of P-glycoprotein (P-gp) has also been intensively studied (Pal & Mitra, 2006), since both participate significantly in multidrug resistance fenotype of many tumours. Our recently-published results show that HY could be a substrate of another ABC-transporting protein, the BCRP (ABCG2) (Jendželovský et al., 2009). We demonstrated that HY affects the expression of these proteins without activation as well.

3.3 The impact of Akt pathway on breast cancer therapy

Akt kinases are downstream components of PI3K derived signals from receptor tyrosine kinases (RTK). It is also the major convergence point for RTK signalling in breast cancer. Several studies have found Akt2 to be amplified or overexpressed at the mRNA level in various tumour cell lines (Miwa et al., 1996) and in a number of human malignancies, such as colon, pancreatic and breast cancers (Bacus et al., 2002; Roy et al., 2002). However, activation of Akt1, Akt2 and Akt3 by phosphorylation appears to be more clinically relevant than detection of Akt2 amplification or overexpression (Cicenas et al., 2005).

Generally, Akt kinase can regulate the proliferation, metabolism as well as survival of cancer cells by modulation of various signalling molecules. The role of Akt protein in surviving cells through inhibition of apoptotic protein suggests that Akt activity may influence the sensitivity of tumour cells to chemotherapy. There have been many studies showing the correlation between chemoresistance and level of phosforylation of Akt in tumours. In their study Cicenas et al. (2005) found that high levels of phosphorylated Akt correlated with poor prognosis in primary breast cancer, and the significance of this correlation increased in the subset of patients with HER2 overexpressing tumours. Moreover phosphorylated Akt contributes to the development of breast cancer, so inhibiting the phosphorylation process could provide a new therapeutic approach (Kucab et al., 2005).

Important data about the role of Akt in cancer cell motility were produced in the study by Yoeli-Lerner et al. (2005), where activation of Akt inhibited carcinoma migration and invasion by breast cancer cells. Their results indicate that Akt can promote tumour progression through increased cell survival mechanism, and it can block breast cancer cell motility and invasion by a mechanism that depends, at least in part, on the nuclear factor of activated T-cells.

The Ras cascade as well as Akt pathways have a major impact on regulation of apoptosis, and moreover they are mutually linked (McCubrey et al., 2006). Both Erk1/2 and EGFR-PI3K-Akt pathways seem to be involved in cellular survival after PDT. The effect of PDT is associated with inactivation of the EGFR-PI3K-Akt pathway. Since EGFR inhibitors and PDT act synergistically, this combination is highly relevant for clinical use (Martinez-Carpio & Trelles, 2010).

Molecule	Modifications	Cell line	References
Akt	inhibition	HT-29	Sačková et al., 2006
	activation/ inhibition	human dermal fibroblasts	Schieke et al., 2004
	activation	BA, BT-474, NIH 3T3, MCF-7	Bozkulak et al., 2007, Zhuang and Kochevar, 2003, Ferenc et al., 2010
	depletion	SKBR-3	Solár et al., 2011
Erk	irreversible inhibition	A431, HaCaT, L929, HeLa	Assefa et al., 1999
	moderate attenuation	human dermal fibroblasts	Schieke et al., 2004
	insignificant modulation	LY-R	Xue et al., 1999
	transient activation	LFS087, GM38A	Tong et al., 2002
	no effect	HaCaT	Klotz et al., 1998
	inhibition /depletion	NCTC 2544	Silva et al., 2010
	activation	MCF-7	Ferenc et al., 2010
	depletion	SKBR-3	Solár et al., 2011

Table 1. Regulation of Akt and Erk by PDT.

A downstream event in the mitogenic Ras pathway is Erk activation through binding of ligands to extracellular growth factor receptors involved in regulation of growth and cell cycle progression. The Ras/Raf/Erk activation pathway can promote opposite prosurvival or anti-proliferative cellular responses, such as apoptosis and autophagy. This wide variety of processes triggered by the activation of a single pathway depends on the timing, duration and strength of activation, on subcellular localization and on the presence of ROS (Cagnol & Chambard, 2010). It is known that ROS induce activation of Ras cascade with increased Erk1/2 activity in various type of cells as a consequence of oxidative stress (Conde de la Rosa et al., 2006).

Available data suggest that the photooxidative stress induced by PDT may modulate Erk activity as does other ROS such as H_2O_2, which is produced in a variety of tumour cell lines by 1,3-dibutyl-2-thiooxoimidazolidine- 4,5-dione (Wong et al., 2010). Decreased phosphorylation status of Akt at Ser 473 without change in Akt level in MCF-7 and MDA-MB-231 cell lines, was observed after application of GE (Chinni et al., 2003). Moreover, GE eliminated irradiation-induced activation of Akt and Erk1/2 (Akimoto et al., 2001).

Application of GE or HY-PDT alone in the study by Ferenc et al. (2010) demonstrated both types of reaction; stimulated Akt and Erk1/2 phosphorylation in MCF-7 cells as well as no effect (Erk1/2; PDT) or even dephosphorylation in MDA-MB-231 cells. Moreover pre-treatment with GE prior to PDT led to suppression of phosphorylation status of Akt and Erk1/2 in both cell lines. Furthermore, Akt protein levels depleted after HY-PDT with GE pre-treatment did not correlated well with mRNA level, which was unaffected. Theoretically, post-translation modification of Akt and Erk1/2 could be partly responsible for effective reduction of proliferarion and clonogenic ability as well as induction of apoptosis recorded in breast adenocarcinoma cells (Ferenc et al., 2010).

One interesting fact revealed in another study (Solár et al., 2011) was a drop in Akt and Erk1/2 activity after elevated oxidative stress achieved by high dose of HY-PDT. Using such high oxidative stress, the upstream molecular target of Erk kinase could be damaged, which might as a final result prevent activation of Erk protein (Lee et al., 2006).

It is well recognized that the majority of cancer-related deaths, including those from breast cancer, is caused by metastatic diseases. To date many new genes and signal pathways involved in this process have been identified. Some genes hold great promise as potential drug targets. Reactivation of metastasis-suppressor genes and their signal pathways such as MKK/JNK, PTEN/Akt and NDRG/ATF is also a rational strategy (Iiizumi et al., 2008).

In accord with the PTEN studies undertaken in the last decade, we would like to point out the very important role of lipid phosphatase in suppression of tumour growth. One of the functions of this tumour suppressor protein is related to negative control of the PI3K/Akt signalling pathway, through dephosphorylation of phosphatidilinositol 3,4,5-triphosphate. Dave et al. (2005) detected induction of apoptosis with elevation of PTEN gene expression in the MCF-7 cell line after application of GE. Furthermore, induced programmed cell death was blocked by using PTEN siRNA. DeGraffenried et al. (2004) observed an interesting result when they detected increased levels of Akt phosphorylation after inhibition of PTEN gene expression. These results were also confirmed by Kikuno et al. (2008) when the elevation of PTEN expression caused silencing of Akt activity. In this regard, significant increases in PTEN expression (MDA-MB-231) and PTEN protein levels have been recorded, and simultaneously decreased phosphorylation of Ser380, Thr382 and Thr383 (important for PTEN protein opening, its translocation to membrane structure

and inhibition of PI3K) has been found after PDT with GE pre-treatment (Ferenc, unpublished data).

3.4 HER2 and photodynamic therapy

An alternative form of treatment, at least for chest wall recurrence of breast carcinoma, is PDT. Allison et al. (2001) succeeded in using PDT to control recurrent breast cancer that had failed to respond to conventional therapy. PDT offers patients with chest wall progression a treatment option with an excellent clinical response and allows opportunities for good long-term local tumour control (Cuenca et al., 2004). One experimental study with ALA-PDT resulted in the downregulation of EGFR mRNA as well as protein levels in a treatment-cycle and light-dose dependent manner in CL1-5, A375 and MDA-MB-231 cells (Tsai et al., 2009). Our recent study showed a decline in HER2 mRNA levels a short time after photoactivation of HY in breast adenocarcinoma cell lines, but no changes in HER2 mRNA were found in dark conditions (Solár et al., 2011). Furthermore, we have also demonstrated HY-PDT mediated degradation of HER2 receptor *via* lysosomal activity (Kovaľ et al., 2010). The efficacy of PDT may be increased using combinations of PDT and anti-VEGF antibody (Bhuvaneswari et al., 2007), or of PDT and EGFR inhibitor (Kovaľ et al., 2010; Weyergang et al., 2008), or using a triple combination of PDT + VEGF inhibitor + EGFR inhibitor. More investigations in animal models to evaluate the efficacy and safety of these combinations are needed (Martinez-Carpio & Trelles, 2010).

4. Conclusion

The aim of this chapter was to summarize the current therapeutic approaches to breast cancer with regard to alternative methods such as PDT. Although great advances have been made during the last 20 years in the treatment of breast cancer and the number of deaths has fallen since the late 1980s, no significant improvement in the survival rates of patients with distant metastases have been observed. The inability to inhibit the resistance of cancer cells, or the development of metastases that may result in the death of the patient represent the principal problems linked with the management of breast cancers. PDT is a relatively new method used for destruction of cutaneous malignancies, but it has been found to be highly efficient against recurrent breast cancer cells. Painless and repeatable treatment is one of the benefits accompanying PDT, which may be used with other regimens or as a single therapy. Significant results in tumour therapies are rarely achieved by the application of a single therapeutic method, and combinations of variable approaches with different mechanisms of action are commonly more efficient. For example, the conjunction of PDT with pharmacological modulators of signalling pathways can either enhance injury of malignant cells, or protect surrounding normal cells.

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6. References

Agostinis, P., Vandenbogaerde, A., Donella-Deana, A., Pinna, L.A., Lee, K.T., Goris, J., Merlevede, W., Vandenheede, J.R. & De Witte, P. (1995). Photosensitized inhibition of growth factor-regulated protein kinases by hypericin. *Biochem Pharmacol*, Vol.49, No.11, pp. 1615-1622. ISSN 0006-2952

Agostinis, P., Vantieghem, A., Merlevede, W. & de Witte, P.A. (2002). Hypericin in cancer treatment: more light on the way. *Int J Biochem Cell Biol*, Vol.34, No.3, pp. 221-241. ISSN 1357-2725

Ahmad, N. & Mukhtar, H. (2000). Mechanism of photodynamic therapy-induced cell death. *Methods Enzymol*, Vol.319, pp. 342-358. ISSN 0076-6879

Akimoto, T., Nonaka, T., Ishikawa, H., Sakurai, H., Saitoh, J.I., Takahashi, T. & Mitsuhashi, N. (2001). Genistein, a tyrosine kinase inhibitor, enhanced radiosensitivity in human esophageal cancer cell lines in vitro: possible involvement of inhibition of survival signal transduction pathways. *Int J Radiat Oncol Biol Phys*, Vol.50, No.1, pp. 195-201. ISSN 0360-3016

Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M. & Fukami, Y. (1987). Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem*, Vol.262, No.12, pp. 5592-5595. ISSN 0021-9258

Allison, R., Mang, T., Hewson, G., Snider, W. & Dougherty, D. (2001). Photodynamic therapy for chest wall progression from breast carcinoma is an underutilized treatment modality. *Cancer*, Vol.91, No.1, pp. 1-8. ISSN 0008-543X

Almeida, R.D., Manadas, B.J., Carvalho, A.P. & Duarte, C.B. (2004). Intracellular signaling mechanisms in photodynamic therapy. *Biochim Biophys Acta*, Vol.1704, No.2, pp. 59-86. ISSN 0006-3002

Assefa, Z., Vantieghem, A., Declercq, W., Vandenabeele, P., Vandenheede, J.R., Merlevede, W., de Witte, P. & Agostinis, P. (1999). The activation of the c-Jun N-terminal kinase and p38 mitogen-activated protein kinase signaling pathways protects HeLa cells from apoptosis following photodynamic therapy with hypericin. *J Biol Chem*, Vol.274, No.13, pp. 8788-8796. ISSN 0021-9258

Bacus, S.S., Altomare, D.A., Lyass, L., Chin, D.M., Farrell, M.P., Gurova, K., Gudkov, A. & Testa, J.R. (2002). AKT2 is frequently upregulated in HER-2/neu-positive breast cancers and may contribute to tumor aggressiveness by enhancing cell survival. *Oncogene*, Vol.21, No.22, pp. 3532-3540. ISSN 0950-9232

Bedard, P.L. & Cardoso, F. (2008). Recent advances in adjuvant systemic therapy for early-stage breast cancer. *Ann Oncol*, Vol.19, Suppl 5, pp. v122-127. ISSN 1569-8041

Belletti, B., Vaidya, J.S., D'Andrea, S., Entschladen, F., Roncadin, M., Lovat, F., Berton, S., Perin, T., Candiani, E., Reccanello, S., Veronesi, A., Canzonieri, V., Trovo, M.G., Zaenker, K.S., Colombatti, A., Baldassarre, G. & Massarut, S. (2008). Targeted intraoperative radiotherapy impairs the stimulation of breast cancer cell proliferation and invasion caused by surgical wounding. *Clin Cancer Res*, Vol.14, No.5, pp. 1325-1332. ISSN 1078-0432

Berg, K., Madslien, K., Bommer, J.C., Oftebro, R., Winkelman, J.W. & Moan, J. (1991). Light induced relocalization of sulfonated meso-tetraphenylporphines in NHIK 3025 cells and effects of dose fractionation. *Photochem Photobiol*, Vol.53, No.2, pp. 203-210. ISSN 0031-8655

Berg, K., Selbo, P.K., Weyergang, A., Dietze, A., Prasmickaite, L., Bonsted, A., Engesaeter, B.O., Angell-Petersen, E., Warloe, T., Frandsen, N. & Hogset, A. (2005). Porphyrin-related photosensitizers for cancer imaging and therapeutic applications. *J Microsc*, Vol.218, No.Pt 2, pp. 133-147. ISSN 0022-2720

Berry, D.A., Cronin, K.A., Plevritis, S.K., Fryback, D.G., Clarke, L., Zelen, M., Mandelblatt, J.S., Yakovlev, A.Y., Habbema, J.D. & Feuer, E.J. (2005). Effect of screening and adjuvant therapy on mortality from breast cancer. *N Engl J Med*, Vol.353, No.17, pp. 1784-1792. ISSN 1533-4406

Beslija, S., Bonneterre, J., Burstein, H., Cocquyt, V., Gnant, M., Goodwin, P., Heinemann, V., Jassem, J., Kostler, W.J., Krainer, M., Menard, S., Petit, T., Petruzelka, L., Possinger, K., Schmid, P., Stadtmauer, E., Stockler, M., Van Belle, S., Vogel, C., Wilcken, N., Wiltschke, C., Zielinski, C.C. & Zwierzina, H. (2007). Second consensus on medical treatment of metastatic breast cancer. *Ann Oncol*, Vol.18, No.2, pp. 215-225. ISSN 0923-7534

Bhuvaneswari, R., Yuen, G.Y., Chee, S.K. & Olivo, M. (2007). Hypericin-mediated photodynamic therapy in combination with Avastin (bevacizumab) improves tumor response by downregulating angiogenic proteins. *Photochem Photobiol Sci*, Vol.6, No.12, pp. 1275-1283. ISSN 1474-905X

Blank, M., Mandel, M., Hazan, S., Keisari, Y. & Lavie, G. (2001). Anti-cancer activities of hypericin in the dark. *Photochem Photobiol*, Vol.74, No.2, pp. 120-125. ISSN 0031-8655

Blank, M., Lavie, G., Mandel, M., Hazan, S., Orenstein, A., Meruelo, D. & Keisari, Y. (2004). Antimetastatic activity of the photodynamic agent hypericin in the dark. *Int J Cancer*, Vol.111, No.4, pp. 596-603. ISSN 0020-7136

Bozkulak, O., Wong, S., Luna, M., Ferrario, A., Rucker, N., Gulsoy, M. & Gomer, C.J. (2007). Multiple components of photodynamic therapy can phosphorylate Akt. *Photochem Photobiol*, Vol.83, No.5, pp. 1029-1033. ISSN 0031-8655

Bryant, H.E., Schultz, N., Thomas, H.D., Parker, K.M., Flower, D., Lopez, E., Kyle, S., Meuth, M., Curtin, N.J. & Helleday, T. (2005). Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature*, Vol.434, No.7035, pp. 913-917. ISSN 1476-4687

Buytaert, E., Callewaert, G., Hendrickx, N., Scorrano, L., Hartmann, D., Missiaen, L., Vandenheede, J.R., Heirman, I., Grooten, J. & Agostinis, P. (2006a). Role of endoplasmic reticulum depletion and multidomain proapoptotic BAX and BAK proteins in shaping cell death after hypericin-mediated photodynamic therapy. *Faseb J*, Vol.20, No.6, pp. 756-758. ISSN 1530-6860

Buytaert, E., Callewaert, G., Vandenheede, J.R. & Agostinis, P. (2006b). Deficiency in apoptotic effectors Bax and Bak reveals an autophagic cell death pathway initiated by photodamage to the endoplasmic reticulum. *Autophagy*, Vol.2, No.3, pp. 238-240. ISSN 1554-8627

Buytaert, E., Dewaele, M. & Agostinis, P. (2007). Molecular effectors of multiple cell death pathways initiated by photodynamic therapy. *Biochim Biophys Acta*, Vol.1776, No.1, pp. 86-107. ISSN 0006-3002

Buytaert, E., Matroule, J.Y., Durinck, S., Close, P., Kocanova, S., Vandenheede, J.R., de Witte, P.A., Piette, J. & Agostinis, P. (2008). Molecular effectors and modulators of hypericin-mediated cell death in bladder cancer cells. *Oncogene*, Vol.27, No.13, pp.1916-1929. ISSN 0950-9232

Cagnol, S. & Chambard, J.C. (2010). ERK and cell death: mechanisms of ERK-induced cell death--apoptosis, autophagy and senescence. *Febs J*, Vol.277, No.1, pp. 2-21. ISSN 1742-4658

Cardoso, F., Van't Veer, L., Rutgers, E., Loi, S., Mook, S. & Piccart-Gebhart, M.J. (2008). Clinical application of the 70-gene profile: the MINDACT trial. *J Clin Oncol*, Vol.26, No.5, pp. 729-735. ISSN 1527-7755

Cicenas, J., Urban, P., Vuaroqueaux, V., Labuhn, M., Kung, W., Wight, E., Mayhew, M., Eppenberger, U. & Eppenberger-Castori, S. (2005). Increased level of phosphorylated akt measured by chemiluminescence-linked immunosorbent assay is a predictor of poor prognosis in primary breast cancer overexpressing ErbB-2. *Breast Cancer Res*, Vol.7, No.4, pp. R394-401. ISSN 1465-542X

Conde de la Rosa, L., Schoemaker, M.H., Vrenken, T.E., Buist-Homan, M., Havinga, R., Jansen, P.L. & Moshage, H. (2006). Superoxide anions and hydrogen peroxide induce hepatocyte death by different mechanisms: involvement of JNK and ERK MAP kinases. *J Hepatol*, Vol.44, No.5, pp. 918-929. ISSN 0168-8278

Constantinou, A.I., Kamath, N. & Murley, J.S. (1998). Genistein inactivates bcl-2, delays the G2/M phase of the cell cycle, and induces apoptosis of human breast adenocarcinoma MCF-7 cells. *Eur J Cancer*, Vol.34, No.12, pp. 1927-1934. ISSN 0959-8049

Couldwell, W.T., Gopalakrishna, R., Hinton, D.R., He, S., Weiss, M.H., Law, R.E. & Apuzzo, M.L. (1994). Hypericin: a potential antiglioma therapy. *Neurosurgery*, Vol.35, No.4, pp. 705-709; discussion 709-710. ISSN 0148-396X

Cuenca, R.E., Allison, R.R., Sibata, C. & Downie, G.H. (2004). Breast cancer with chest wall progression: treatment with photodynamic therapy. *Ann Surg Oncol*, Vol.11, No.3, pp. 322-327. ISSN 1068-9265

Čavarga, I., Brezáni, P., Fedoročko, P., Miškovský, P., Bobrov, N., Longauer, F., Rybárová, S., Miroššay, L. & Štubňa, J. (2005). Photoinduced antitumour effect of hypericin can be enhanced by fractionated dosing. *Phytomedicine*, Vol.12, No.9, pp. 680-683. ISSN 0944-7113

Dave, B., Eason, R.R., Till, S.R., Geng, Y., Velarde, M.C., Badger, T.M. & Simmen, R.C. (2005). The soy isoflavone genistein promotes apoptosis in mammary epithelial cells by inducing the tumor suppressor PTEN. *Carcinogenesis*, Vol.26, No.10, pp. 1793-1803. ISSN 0143-3334

DeGraffenried, L.A., Fulcher, L., Friedrichs, W.E., Grunwald, V., Ray, R.B. & Hidalgo, M. (2004). Reduced PTEN expression in breast cancer cells confers susceptibility to inhibitors of the PI3 kinase/Akt pathway. *Ann Oncol*, Vol.15, No.10, pp. 1510-1516. ISSN 0923-7534

Delaey, E., Vandenbogaerde, A., Merlevede, W. & de Witte, P. (2000). Photocytotoxicity of hypericin in normoxic and hypoxic conditions. *J Photochem Photobiol B*, Vol.56, No.1, pp. 19-24. ISSN 1011-1344

Demaria, S. & Formenti, S.C. (2007). Sensors of ionizing radiation effects on the immunological microenvironment of cancer. *Int J Radiat Biol*, Vol.83, No.11-12, pp. 819-825. ISSN 0955-3002

du Manoir, J.M., Francia, G., Man, S., Mossoba, M., Medin, J.A., Viloria-Petit, A., Hicklin, D.J., Emmenegger, U. & Kerbel, R.S. (2006). Strategies for delaying or treating in

vivo acquired resistance to trastuzumab in human breast cancer xenografts. *Clin Cancer Res*, Vol.12, No.3 Pt 1, pp. 904-916. ISSN 1078-0432

Ferenc, P., Solár, P., Kleban, J., Mikeš, J. & Fedoročko, P. (2010). Down-regulation of Bcl-2 and Akt induced by combination of photoactivated hypericin and genistein in human breast cancer cells. *J Photochem Photobiol B*, Vol.98, No.1, pp. 25-34. ISSN 1873-2682

Fernando, I.N. (2000). The role of radiotherapy in patients undergoing mastectomy for carcinoma of the breast. *Clin Oncol (R Coll Radiol)*, Vol.12, No.3, pp. 158-165. ISSN 0936-6555

Festjens, N., Vanden Berghe, T. & Vandenabeele, P. (2006). Necrosis, a well-orchestrated form of cell demise: signalling cascades, important mediators and concomitant immune response. *Biochim Biophys Acta*, Vol.1757, No.9-10, pp. 1371-1387. ISSN 0006-3002

Fisher, B., Costantino, J.P., Redmond, C.K., Fisher, E.R., Wickerham, D.L. & Cronin, W.M. (1994). Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14. *J Natl Cancer Inst*, Vol.86, No.7, pp. 527-537. ISSN 0027-8874

Formenti, S.C. & Demaria, S. (2008). Local control by radiotherapy: is that all there is? *Breast Cancer Res*, Vol.10, No.6, pp. 215. ISSN 1465-542X

Forrest, A.P. (1982). Beatson: hormones and the management of breast cancer. *J R Coll Surg Edinb*, Vol.27, No.5, pp. 253-263. ISSN 0035-8835

Fritsch, C. & Ruzicka, T. (2006). Fluorescence diagnosis and photodynamic therapy in dermatology from experimental state to clinic standard methods. *J Environ Pathol Toxicol Oncol*, Vol.25, No.1-2, pp. 425-439. ISSN 0731-8898

Gilbert, F.J. (2008). Breast cancer screening in high risk women. *Cancer Imaging*, Vol.8, Spec No A, pp. S6-9. ISSN 1470-7330

Hadjur, C., Richard, M.J., Parat, M.O., Jardon, P. & Favier, A. (1996). Photodynamic effects of hypericin on lipid peroxidation and antioxidant status in melanoma cells. *Photochem Photobiol*, Vol.64, No.2, pp. 375-381. ISSN 0031-8655

Haffty, B.G., Hauser, A., Choi, D.H., Parisot, N., Rimm, D., King, B. & Carter, D. (2004). Molecular markers for prognosis after isolated postmastectomy chest wall recurrence. *Cancer*, Vol.100, No.2, pp. 252-263. ISSN 0008-543X

Harada, N. (1997). Aberrant expression of aromatase in breast cancer tissues. *J Steroid Biochem Mol Biol*, Vol.61, No.3-6, pp. 175-184. ISSN 0960-0760

Hellerhoff, K. (2010). [Digital breast tomosynthesis: technical principles, current clinical relevance and future perspectives]. *Radiologe*, Vol.50, No.11, pp. 991-998. ISSN 1432-2102

Hendrickx, N., Volanti, C., Moens, U., Seternes, O.M., de Witte, P., Vandenheede, J.R., Piette, J. & Agostinis, P. (2003). Up-regulation of cyclooxygenase-2 and apoptosis resistance by p38 MAPK in hypericin-mediated photodynamic therapy of human cancer cells. *J Biol Chem*, Vol.278, No.52, pp. 52231-52239. ISSN 0021-9258

Hopper, C. (1996). The role of photodynamic therapy in the management of oral cancer and precancer. *Eur J Cancer B Oral Oncol*, Vol.32B, No.2, pp. 71-72. ISSN 0964-1955

Huygens, A., Kamuhabwa, A.R., Van Laethem, A., Roskams, T., Van Cleynenbreugel, B., Van Poppel, H., Agostinis, P. & De Witte, P.A. (2005). Enhancing the photodynamic effect of hypericin in tumour spheroids by fractionated light delivery in

combination with hyperoxygenation. *Int J Oncol*, Vol.26, No.6, pp. 1691-1697. ISSN 1019-6439

Chakraborty, M., Abrams, S.I., Camphausen, K., Liu, K., Scott, T., Coleman, C.N. & Hodge, J.W. (2003). Irradiation of tumor cells up-regulates Fas and enhances CTL lytic activity and CTL adoptive immunotherapy. *J Immunol*, Vol.170, No.12, pp. 6338-6347. ISSN 0022-1767

Chaloupka, R., Obsil, T., Plasek, J. & Sureau, F. (1999). The effect of hypericin and hypocrellin-A on lipid membranes and membrane potential of 3T3 fibroblasts. *Biochim Biophys Acta*, Vol.1418, No.1, pp. 39-47. ISSN 0006-3002

Chan, P.S., Koon, H.K., Wu, Z.G., Wong, R.N., Lung, M.L., Chang, C.K. & Mak, N.K. (2009). Role of p38 MAPKs in hypericin photodynamic therapy-induced apoptosis of nasopharyngeal carcinoma cells. *Photochem Photobiol*, Vol.85, No.5, pp. 1207-1217. ISSN 0031-8655

Chan, S., Scheulen, M.E., Johnston, S., Mross, K., Cardoso, F., Dittrich, C., Eiermann, W., Hess, D., Morant, R., Semiglazov, V., Borner, M., Salzberg, M., Ostapenko, V., Illiger, H.J., Behringer, D., Bardy-Bouxin, N., Boni, J., Kong, S., Cincotta, M. & Moore, L. (2005). Phase II study of temsirolimus (CCI-779), a novel inhibitor of mTOR, in heavily pretreated patients with locally advanced or metastatic breast cancer. *J Clin Oncol*, Vol.23, No.23, pp. 5314-5322. ISSN 0732-183X

Chinni, S.R., Alhasan, S.A., Multani, A.S., Pathak, S. & Sarkar, F.H. (2003). Pleotropic effects of genistein on MCF-7 breast cancer cells. *Int J Mol Med*, Vol.12, No.1, pp. 29-34. ISSN 1107-3756

Chumsri, S., Howes, T., Bao, T., Sabnis, G. & Brodie, A. (2011). Aromatase, aromatase inhibitors, and breast cancer. *J Steroid Biochem Mol Biol*, Vol.125, No.1-2, pp. 13-22.

Iiizumi, M., Liu, W., Pai, S.K., Furuta, E. & Watabe, K. (2008). Drug development against metastasis-related genes and their pathways: a rationale for cancer therapy. *Biochim Biophys Acta*, Vol.1786, No.2, pp. 87-104. ISSN 0006-3002

Iwasaki, M., Inoue, M., Otani, T., Sasazuki, S., Kurahashi, N., Miura, T., Yamamoto, S. & Tsugane, S. (2008). Plasma isoflavone level and subsequent risk of breast cancer among Japanese women: a nested case-control study from the Japan Public Health Center-based prospective study group. *J Clin Oncol*, Vol.26, No.10, pp. 1677-1683. ISSN 1527-7755

Jacobson, J.M., Feinman, L., Liebes, L., Ostrow, N., Koslowski, V., Tobia, A., Cabana, B.E., Lee, D., Spritzler, J. & Prince, A.M. (2001). Pharmacokinetics, safety, and antiviral effects of hypericin, a derivative of St. John's wort plant, in patients with chronic hepatitis C virus infection. *Antimicrob Agents Chemother*, Vol.45, No.2, pp. 517-524. ISSN 0066-4804

James, C.R., Quinn, J.E., Mullan, P.B., Johnston, P.G. & Harkin, D.P. (2007). BRCA1, a potential predictive biomarker in the treatment of breast cancer. *Oncologist*, Vol.12, No.2, pp. 142-150. ISSN 1083-7159

Jang, E.R., Lim, S.J., Lee, E.S., Jeong, G., Kim, T.Y., Bang, Y.J. & Lee, J.S. (2004). The histone deacetylase inhibitor trichostatin A sensitizes estrogen receptor alpha-negative breast cancer cells to tamoxifen. *Oncogene*, Vol.23, No.9, pp. 1724-1736. ISSN 0950-9232

Jendželovský, R., Mikeš, J., Kovaľ, J., Souček, K., Procházková, J., Kello, M., Sačková, V., Hofmanová, J., Kozubík, A. & Fedoročko, P. (2009). Drug efflux transporters, MRP1

and BCRP, affect the outcome of hypericin-mediated photodynamic therapy in HT-29 adenocarcinoma cells. *Photochem Photobiol Sci*, Vol.8, No.12, pp. 1716-1723. ISSN 1474-9092

Jichlinski, P. & Leisinger, H.J. (2005). Fluorescence cystoscopy in the management of bladder cancer: a help for the urologist! *Urol Int*, Vol.74, No.2, pp. 97-101. ISSN 0042-1138

Johnson, S.A. & Pardini, R.S. (1998). Antioxidant enzyme response to hypericin in EMT6 mouse mammary carcinoma cells. *Free Radic Biol Med*, Vol.24, No.5, pp. 817-826. ISSN 0891-5849

Jordan, V.C. (1995). Tamoxifen: toxicities and drug resistance during the treatment and prevention of breast cancer. *Annu Rev Pharmacol Toxicol*, Vol.35, pp. 195-211. ISSN 0362-1642

Kaščáková, S., Naďová, Z., Mateasik, A., Mikeš, J., Huntošová, V., Refregiers, M., Sureau, F., Maurizot, J.C., Miškovský, P. & Jancura, D. (2008). High level of low-density lipoprotein receptors enhance hypericin uptake by U-87 MG cells in the presence of LDL. *Photochem Photobiol*, Vol.84, No.1, pp. 120-127. ISSN 0031-8655

Kessel, D., Luo, Y., Deng, Y. & Chang, C.K. (1997). The role of subcellular localization in initiation of apoptosis by photodynamic therapy. *Photochem Photobiol*, Vol.65, No.3, pp. 422-426. ISSN 0031-8655

Kikuno, N., Shiina, H., Urakami, S., Kawamoto, K., Hirata, H., Tanaka, Y., Majid, S., Igawa, M. & Dahiya, R. (2008). Genistein mediated histone acetylation and demethylation activates tumor suppressor genes in prostate cancer cells. *Int J Cancer*, Vol.123, No.3, pp. 552-560. ISSN 1097-0215

Klotz, L.O., Fritsch, C., Briviba, K., Tsacmacidis, N., Schliess, F. & Sies, H. (1998). Activation of JNK and p38 but not ERK MAP kinases in human skin cells by 5-aminolevulinate-photodynamic therapy. *Cancer Res*, Vol.58, No.19, pp. 4297-4300. ISSN 0008-5472

Kovaľ, J., Mikeš, J., Jendželovský, R., Kello, M., Solár, P. & Fedoročko, P. (2010). Degradation of HER2 receptor through hypericin-mediated photodynamic therapy. *Photochem Photobiol*, Vol.86, No.1, pp. 200-205. ISSN 1751-1097

Kucab, J.E., Lee, C., Chen, C.S., Zhu, J., Gilks, C.B., Cheang, M., Huntsman, D., Yorida, E., Emerman, J., Pollak, M. & Dunn, S.E. (2005). Celecoxib analogues disrupt Akt signaling, which is commonly activated in primary breast tumours. *Breast Cancer Res*, Vol.7, No.5, pp. R796-807. ISSN 1465-542X

Kuliková, L., Mikeš, J., Hýždalová, M., Palumbo, G. & Fedoročko, P. (2010). NF-kappaB is not directly responsible for photoresistance induced by fractionated light delivery in HT-29 colon adenocarcinoma cells. *Photochem Photobiol*, Vol.86, No.6, pp. 1285-1293. ISSN 1751-1097

Lavie, G., Meruelo, D., Aroyo, K. & Mandel, M. (2000). Inhibition of the CD8+ T cell-mediated cytotoxicity reaction by hypericin: potential for treatment of T cell-mediated diseases. *Int Immunol*, Vol.12, No.4, pp. 479-486. ISSN 0953-8178

Ledo, E. & Ledo, A. (2000). Phototherapy, photochemotherapy, and photodynamic therapy: unapproved uses or indications. *Clin Dermatol*, Vol.18, No.1, pp. 77-86. ISSN 0738-081X

Lee, J.S., Kim, S.Y., Kwon, C.H. & Kim, Y.K. (2006). EGFR-dependent ERK activation triggers hydrogen peroxide-induced apoptosis in OK renal epithelial cells. *Arch Toxicol*, Vol.80, No.6, pp. 337-346. ISSN 0340-5761

Lee, W.R., Shen, S.C., Lin, H.Y., Hou, W.C., Yang, L.L. & Chen, Y.C. (2002). Wogonin and fisetin induce apoptosis in human promyeloleukemic cells, accompanied by a decrease of reactive oxygen species, and activation of caspase 3 and Ca(2+)-dependent endonuclease. *Biochem Pharmacol*, Vol.63, No.2, pp. 225-236. ISSN 0006-2952

Leopold, E. (1999). *A Darker Ribbon: Breast Cancer, Women, and their Doctors in the Twentieth Century.*, Beacon Press, ISBN 978-0807065136, Boston

Li, Z., Li, J., Mo, B., Hu, C., Liu, H., Qi, H., Wang, X. & Xu, J. (2008). Genistein induces cell apoptosis in MDA-MB-231 breast cancer cells via the mitogen-activated protein kinase pathway. *Toxicol In Vitro*, Vol.22, No.7, pp. 1749-1753. ISSN 0887-2333

Liu, S., Sugimoto, Y., Kulp, S.K., Jiang, J., Chang, H.L., Park, K.Y., Kashida, Y. & Lin, Y.C. (2002). Estrogenic down-regulation of protein tyrosine phosphatase gamma (PTP gamma) in human breast is associated with estrogen receptor alpha. *Anticancer Res*, Vol.22, No.6C, pp. 3917-3923. ISSN 0250-7005

Lopez-Tarruella, S. & Martin, M. (2009). Recent advances in systemic therapy: advances in adjuvant systemic chemotherapy of early breast cancer. *Breast Cancer Res*, Vol.11, No.2, art.no.204.. ISSN 1465-542X

Lu, Y., Zi, X., Zhao, Y., Mascarenhas, D. & Pollak, M. (2001). Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). *J Natl Cancer Inst*, Vol.93, No.24, pp. 1852-1857. ISSN 0027-8874

Lugade, A.A., Sorensen, E.W., Gerber, S.A., Moran, J.P., Frelinger, J.G. & Lord, E.M. (2008). Radiation-induced IFN-gamma production within the tumor microenvironment influences antitumor immunity. *J Immunol*, Vol.180, No.5, pp. 3132-3139. ISSN 0022-1767

Marchal, S., Francois, A., Dumas, D., Guillemin, F. & Bezdetnaya, L. (2007). Relationship between subcellular localisation of Foscan and caspase activation in photosensitised MCF-7 cells. *Br J Cancer*, Vol.96, No.6, pp. 944-951. ISSN 0007-0920

Marini, H., Minutoli, L., Polito, F., Bitto, A., Altavilla, D., Atteritano, M., Gaudio, A., Mazzaferro, S., Frisina, A., Frisina, N., Lubrano, C., Bonaiuto, M., D'Anna, R., Cannata, M.L., Corrado, F., Cancellieri, F., Faraci, M., Marini, R., Adamo, E.B., Wilson, S. & Squadrito, F. (2008). OPG and sRANKL serum concentrations in osteopenic, postmenopausal women after 2-year genistein administration. *J Bone Miner Res*, Vol.23, No.5, pp. 715-720. ISSN 1523-4681

Martinez-Carpio, P.A. & Trelles, M.A. (2010). The role of epidermal growth factor receptor in photodynamic therapy: a review of the literature and proposal for future investigation. *Lasers Med Sci*, Vol.25, No.6, pp. 767-771. ISSN 1435-604X

Matsumura, S., Wang, B., Kawashima, N., Braunstein, S., Badura, M., Cameron, T.O., Babb, J.S., Schneider, R.J., Formenti, S.C., Dustin, M.L. & Demaria, S. (2008). Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells. *J Immunol*, Vol.181, No.5, pp. 3099-3107. ISSN 1550-6606

McCubrey, J.A., Steelman, L.S., Abrams, S.L., Lee, J.T., Chang, F., Bertrand, F.E., Navolanic, P.M., Terrian, D.M., Franklin, R.A., D'Assoro, A.B., Salisbury, J.L., Mazzarino, M.C., Stivala, F. & Libra, M. (2006). Roles of the RAF/MEK/ERK and PI3K/PTEN/AKT pathways in malignant transformation and drug resistance. *Adv Enzyme Regul*, Vol.46, No.1, pp. 249-279. ISSN 0065-2571

Messina, M., McCaskill-Stevens, W. & Lampe, J.W. (2006). Addressing the soy and breast cancer relationship: review, commentary, and workshop proceedings. *J Natl Cancer Inst*, Vol.98, No.18, pp. 1275-1284. ISSN 1460-2105

Miadoková, E., Chalupa, I., Vlčková, V., Ševčovičová, A., Naďová, S., Kopasková, M., Hercegová, A., Gašperová, P., Alfoldiová, L., Komjatiová, M., Czanyiová, Z., Gálová, E., Čellárová, E. & Vlček, D. (2009). Genotoxicity and antigenotoxicity evaluation of non-photoactivated hypericin. *Phytother Res*, Vol.24, No.1, pp. 90-95. ISSN 1099-1573

Mikeš, J., Kovaľ, J., Jendželovský, R., Sačková, V., Uhrínová, I., Kello, M., Kuliková, L. & Fedoročko, P. (2009). The role of p53 in the efficiency of photodynamic therapy with hypericin and subsequent long-term survival of colon cancer cells. *Photochem Photobiol Sci*, Vol.8, No.11, pp. 1558-1567. ISSN 1474-9092

Miles, D.W. (2009). Recent advances in systemic therapy. When HER2 is not the target: advances in the treatment of HER2-negative metastatic breast cancer. *Breast Cancer Res*, Vol.11, No.4, pp. 208. ISSN 1465-542X

Miller, K., Wang, M., Gralow, J., Dickler, M., Cobleigh, M., Perez, E.A., Shenkier, T., Cella, D. & Davidson, N.E. (2007). Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med*, Vol.357, No.26, pp. 2666-2676. ISSN 1533-4406

Miller, K.D., Chap, L.I., Holmes, F.A., Cobleigh, M.A., Marcom, P.K., Fehrenbacher, L., Dickler, M., Overmoyer, B.A., Reimann, J.D., Sing, A.P., Langmuir, V. & Rugo, H.S. (2005). Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. *J Clin Oncol*, Vol.23, No.4, pp. 792-799. ISSN 0732-183X

Miwa, W., Yasuda, J., Murakami, Y., Yashima, K., Sugano, K., Sekine, T., Kono, A., Egawa, S., Yamaguchi, K., Hayashizaki, Y. & Sekiya, T. (1996). Isolation of DNA sequences amplified at chromosome 19q13.1-q13.2 including the AKT2 locus in human pancreatic cancer. *Biochem Biophys Res Commun*, Vol.225, No.3, pp. 968-974. ISSN 0006-291X

Moan, J. & Berg, K. (1991). The photodegradation of porphyrins in cells can be used to estimate the lifetime of singlet oxygen. *Photochem Photobiol*, Vol.53, No.4, pp. 549-553. ISSN 0031-8655

Morrow, P.K., Zambrana, F. & Esteva, F.J. (2009). Recent advances in systemic therapy: Advances in systemic therapy for HER2-positive metastatic breast cancer. *Breast Cancer Res*, Vol.11, No.4, art. no.207. ISSN 1465-542X

Muthyala, R.S., Ju, Y.H., Sheng, S., Williams, L.D., Doerge, D.R., Katzenellenbogen, B.S., Helferich, W.G. & Katzenellenbogen, J.A. (2004). Equol, a natural estrogenic metabolite from soy isoflavones: convenient preparation and resolution of R- and S-equols and their differing binding and biological activity through estrogen receptors alpha and beta. *Bioorg Med Chem*, Vol.12, No.6, pp. 1559-1567. ISSN 0968-0896

Nagata, Y., Lan, K.H., Zhou, X., Tan, M., Esteva, F.J., Sahin, A.A., Klos, K.S., Li, P., Monia, B.P., Nguyen, N.T., Hortobagyi, G.N., Hung, M.C. & Yu, D. (2004). PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell*, Vol.6, No.2, pp. 117-127. ISSN 1535-6108

Nagy, P., Friedlander, E., Tanner, M., Kapanen, A.I., Carraway, K.L., Isola, J. & Jovin, T.M. (2005). Decreased accessibility and lack of activation of ErbB2 in JIMT-1, a herceptin-resistant, MUC4-expressing breast cancer cell line. *Cancer Res*, Vol.65, No.2, pp. 473-482. ISSN 0008-5472

Nahta, R., Yu, D., Hung, M.C., Hortobagyi, G.N. & Esteva, F.J. (2006). Mechanisms of disease: understanding resistance to HER2-targeted therapy in human breast cancer. *Nat Clin Pract Oncol*, Vol.3, No.5, pp. 269-280. ISSN 1743-4254

Nguewa, P.A., Fuertes, M.A., Cepeda, V., Alonso, C., Quevedo, C., Soto, M. & Perez, J.M. (2006). Poly(ADP-ribose) polymerase-1 inhibitor 3-aminobenzamide enhances apoptosis induction by platinum complexes in cisplatin-resistant tumor cells. *Med Chem*, Vol.2, No.1, pp. 47-53. ISSN 1573-4064

Niedre, M., Patterson, M.S. & Wilson, B.C. (2002). Direct near-infrared luminescence detection of singlet oxygen generated by photodynamic therapy in cells in vitro and tissues in vivo. *Photochem Photobiol*, Vol.75, No.4, pp. 382-391. ISSN 0031-8655

Nomoto, S., Arao, Y., Horiguchi, H., Ikeda, K. & Kayama, F. (2002). Oestrogen causes G2/M arrest and apoptosis in breast cancer cells MDA-MB-231. *Oncol Rep*, Vol.9, No.4, pp. 773-776. ISSN 1021-335X

Normanno, N., Di Maio, M., De Maio, E., De Luca, A., de Matteis, A., Giordano, A. & Perrone, F. (2005). Mechanisms of endocrine resistance and novel therapeutic strategies in breast cancer. *Endocr Relat Cancer*, Vol.12, No.4, pp. 721-747. ISSN 1351-0088

O'Day, E. & Lal, A. (2010). MicroRNAs and their target gene networks in breast cancer. *Breast Cancer Res*, Vol.12, No.2, art. no.201. ISSN 1465-542X

Obach, R.S. (2000). Inhibition of human cytochrome P450 enzymes by constituents of St. John's Wort, an herbal preparation used in the treatment of depression. *J Pharmacol Exp Ther*, Vol.294, No.1, pp. 88-95. ISSN 0022-3565

Oleinick, N.L., Morris, R.L. & Belichenko, I. (2002). The role of apoptosis in response to photodynamic therapy: what, where, why, and how. *Photochem Photobiol Sci*, Vol.1, No.1, pp. 1-21. ISSN 1474-905X

Olson, J. (2002). *Bathsheba's Breast: Women, Cancer, and History.*, John Hopkins Press, ISBN 978-0801869365, Baltimore

Pal, D. & Mitra, A.K. (2006). MDR- and CYP3A4-mediated drug-herbal interactions. *Life Sci*, Vol.78, No.18, pp. 2131-2145. ISSN 0024-3205

Pavese, J.M., Farmer, R.L. & Bergan, R.C. (2010). Inhibition of cancer cell invasion and metastasis by genistein. *Cancer Metastasis Rev*, Vol.29, No.3, pp. 465-482. ISSN 1573-7233

Pegram, M.D., Pienkowski, T., Northfelt, D.W., Eiermann, W., Patel, R., Fumoleau, P., Quan, E., Crown, J., Toppmeyer, D., Smylie, M., Riva, A., Blitz, S., Press, M.F., Reese, D., Lindsay, M.A. & Slamon, D.J. (2004). Results of two open-label, multicenter phase II studies of docetaxel, platinum salts, and trastuzumab in HER2-positive advanced breast cancer. *J Natl Cancer Inst*, Vol.96, No.10, pp. 759-769. ISSN 1460-2105

Perez, E.A., Suman, V.J., Rowland, K.M., Ingle, J.N., Salim, M., Loprinzi, C.L., Flynn, P.J., Mailliard, J.A., Kardinal, C.G., Krook, J.E., Thrower, A.R., Visscher, D.W. & Jenkins, R.B. (2005). Two concurrent phase II trials of paclitaxel/carboplatin/trastuzumab (weekly or every-3-week schedule) as first-line therapy in women with HER2-

overexpressing metastatic breast cancer: NCCTG study 983252. *Clin Breast Cancer*, Vol.6, No.5, pp. 425-432. ISSN 1526-8209

Plaetzer, K., Kiesslich, T., Oberdanner, C.B. & Krammer, B. (2005). Apoptosis following photodynamic tumor therapy: induction, mechanisms and detection. *Curr Pharm Des*, Vol.11, No.9, pp. 1151-1165. ISSN 1381-6128

Powell, S. (2010). Radiotherapy for breast cancer in the 21st Century. *Breast J*, Vol.16, Suppl 1, pp. S34-38. ISSN 1524-4741

Proskuryakov, S.Y., Konoplyannikov, A.G. & Gabai, V.L. (2003). Necrosis: a specific form of programmed cell death? *Exp Cell Res*, Vol.283, No.1, pp. 1-16. ISSN 0014-4827

Qiang, Y.G., Yow, C.M. & Huang, Z. (2008). Combination of photodynamic therapy and immunomodulation: current status and future trends. *Med Res Rev*, Vol.28, No.4, pp. 632-644. ISSN 0198-6325

Rajah, T.T., Du, N., Drews, N. & Cohn, R. (2009). Genistein in the presence of 17beta-estradiol inhibits proliferation of ERbeta breast cancer cells. *Pharmacology*, Vol.84, No.2, pp. 68-73. ISSN 1423-0313

Rasola, A. & Bernardi, P. (2007). The mitochondrial permeability transition pore and its involvement in cell death and in disease pathogenesis. *Apoptosis*, Vol.12, No.5, pp. 815-833. ISSN 1360-8185

Reddy, G.R., Bhojani, M.S., McConville, P., Moody, J., Moffat, B.A., Hall, D.E., Kim, G., Koo, Y.E., Woolliscroft, M.J., Sugai, J.V., Johnson, T.D., Philbert, M.A., Kopelman, R., Rehemtulla, A. & Ross, B.D. (2006). Vascular targeted nanoparticles for imaging and treatment of brain tumors. *Clin Cancer Res*, Vol.12, No.22, pp. 6677-6686. ISSN 1078-0432

Redmond, R.W. & Gamlin, J.N. (1999). A compilation of singlet oxygen yields from biologically relevant molecules. *Photochem Photobiol*, Vol.70, No.4, pp. 391-475. ISSN 0031-8655

Reed, J.C. (1998). Bcl-2 family proteins. *Oncogene*, Vol.17, No.25, pp. 3225-3236. ISSN 0950-9232

Reits, E.A., Hodge, J.W., Herberts, C.A., Groothuis, T.A., Chakraborty, M., Wansley, E.K., Camphausen, K., Luiten, R.M., de Ru, A.H., Neijssen, J., Griekspoor, A., Mesman, E., Verreck, F.A., Spits, H., Schlom, J., van Veelen, P. & Neefjes, J.J. (2006). Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med*, Vol.203, No.5, pp. 1259-1271. ISSN 0022-1007

Robert, N., Leyland-Jones, B., Asmar, L., Belt, R., Ilegbodu, D., Loesch, D., Raju, R., Valentine, E., Sayre, R., Cobleigh, M., Albain, K., McCullough, C., Fuchs, L. & Slamon, D. (2006). Randomized phase III study of trastuzumab, paclitaxel, and carboplatin compared with trastuzumab and paclitaxel in women with HER-2-overexpressing metastatic breast cancer. *J Clin Oncol*, Vol.24, No.18, pp. 2786-2792. ISSN 1527-7755

Roy, H.K., Olusola, B.F., Clemens, D.L., Karolski, W.J., Ratashak, A., Lynch, H.T. & Smyrk, T.C. (2002). AKT proto-oncogene overexpression is an early event during sporadic colon carcinogenesis. *Carcinogenesis*, Vol.23, No.1, pp. 201-205. ISSN 0143-3334

Sačková, V., Kulíková, L., Mikeš, J., Kleban, J. & Fedoročko, P. (2005). Hypericin-mediated photocytotoxic effect on HT-29 adenocarcinoma cells is reduced by light

fractionation with longer dark pause between two unequal light doses. *Photochem Photobiol*, Vol.81, No.6, pp. 1411-1416. ISSN 0031-8655

Sačková, V., Fedoročko, P., Szilárdiová, B., Mikeš, J. & Kleban, J. (2006). Hypericin-induced phototoxicity is connected with G2/M arrest in HT-29 and S phase arrest in U937 cells. *Photochem Photobiol*, Vol.82, No.5, pp. 1285-1291. ISSN 0031-8655

Shepard, H.M., Jin, P., Slamon, D.J., Pirot, Z. & Maneval, D.C. (2008). Herceptin. *Handb Exp Pharmacol*, No.181, pp. 183-219. ISSN 0171-2004

Schieke, S.M., von Montfort, C., Buchczyk, D.P., Timmer, A., Grether-Beck, S., Krutmann, J., Holbrook, N.J. & Klotz, L.O. (2004). Singlet oxygen-induced attenuation of growth factor signaling: possible role of ceramides. *Free Radic Res*, Vol.38, No.7, pp. 729-737. ISSN 1071-5762

Schinazi, R.F., Chu, C.K., Babu, J.R., Oswald, B.J., Saalmann, V., Cannon, D.L., Eriksson, B.F. & Nasr, M. (1990). Anthraquinones as a new class of antiviral agents against human immunodeficiency virus. *Antiviral Res*, Vol.13, No.5, pp. 265-272. ISSN 0166-3542

Schlotter, C.M., Vogt, U., Allgayer, H. & Brandt, B. (2008). Molecular targeted therapies for breast cancer treatment. *Breast Cancer Res*, Vol.10, No.4, pp. 211. ISSN 1465-542X

Silva, J.N., Galmiche, A., Tome, J.P., Boullier, A., Neves, M.G., Silva, E.M., Capiod, J.C., Cavaleiro, J.A., Santus, R., Maziere, J.C., Filipe, P. & Morliere, P. (2010). Chain-dependent photocytotoxicity of tricationic porphyrin conjugates and related mechanisms of cell death in proliferating human skin keratinocytes. *Biochem Pharmacol*, Vol.80, No.9, pp. 1373-1385. ISSN 1873-2968

Simon, V., Devaux, C., Darmon, A., Donnet, T., Thienot, E., Germain, M., Honnorat, J., Duval, A., Pottier, A., Borghi, E., Levy, L. & Marill, J. (2010). Pp IX silica nanoparticles demonstrate differential interactions with in vitro tumor cell lines and in vivo mouse models of human cancers. *Photochem Photobiol*, Vol.86, No.1, pp. 213-222. ISSN 1751-1097

Solár, P., Ferenc, P., Kovaľ, J., Mikeš, J., Solárová, Z., Hrčková, G., Fulton, B.L. & Fedoročko, P. (2011). Photoactivated hypericin induces downregulation of HER2 gene expression. *Radiat Res*, Vol.175, No.1, pp. 51-56. ISSN 1938-5404

Sparano, J.A. & Paik, S. (2008). Development of the 21-gene assay and its application in clinical practice and clinical trials. *J Clin Oncol*, Vol.26, No.5, pp. 721-728. ISSN 1527-7755

Takemura, T., Ohta, N., Nakajima, S. & Sakata, I. (1989). Critical importance of the triplet lifetime of photosensitizer in photodynamic therapy of tumor. *Photochem Photobiol*, Vol.50, No.3, pp. 339-344. ISSN 0031-8655

Thomas, E.S., Gomez, H.L., Li, R.K., Chung, H.C., Fein, L.E., Chan, V.F., Jassem, J., Pivot, X.B., Klimovsky, J.V., de Mendoza, F.H., Xu, B., Campone, M., Lerzo, G.L., Peck, R.A., Mukhopadhyay, P., Vahdat, L.T. & Roche, H.H. (2007). Ixabepilone plus capecitabine for metastatic breast cancer progressing after anthracycline and taxane treatment. *J Clin Oncol*, Vol.25, No.33, pp. 5210-5217. ISSN 1527-7755

Thong, P.S., Olivo, M., Chin, W.W., Bhuvaneswari, R., Mancer, K. & Soo, K.C. (2009). Clinical application of fluorescence endoscopic imaging using hypericin for the diagnosis of human oral cavity lesions. *Br J Cancer*, Vol.101, No.9, pp. 1580-1584. ISSN 1532-1827

Tong, Z., Singh, G. & Rainbow, A.J. (2002). Sustained activation of the extracellular signal-regulated kinase pathway protects cells from photofrin-mediated photodynamic therapy. *Cancer Res*, Vol.62, No.19, pp. 5528-5535. ISSN 0008-5472

Tsai, T., Ji, H.T., Chiang, P.C., Chou, R.H., Chang, W.S. & Chen, C.T. (2009). ALA-PDT results in phenotypic changes and decreased cellular invasion in surviving cancer cells. *Lasers Surg Med*, Vol.41, No.4, pp. 305-315. ISSN 1096-9101

Utsumi, T., Okuma, M., Kanno, T., Takehara, Y., Yoshioka, T., Fujita, Y., Horton, A.A. & Utsumi, K. (1995). Effect of the antiretroviral agent hypericin on rat liver mitochondria. *Biochem Pharmacol*, Vol.50, No.5, pp. 655-662. ISSN 0006-2952

Vantieghem, A., Xu, Y., Declercq, W., Vandenabeele, P., Denecker, G., Vandenheede, J.R., Merlevede, W., de Witte, P.A. & Agostinis, P. (2001). Different pathways mediate cytochrome c release after photodynamic therapy with hypericin. *Photochem Photobiol*, Vol.74, No.2, pp. 133-142. ISSN 0031-8655

Verheus, M., van Gils, C.H., Keinan-Boker, L., Grace, P.B., Bingham, S.A. & Peeters, P.H. (2007). Plasma phytoestrogens and subsequent breast cancer risk. *J Clin Oncol*, Vol.25, No.6, pp. 648-655. ISSN 1527-7755

von Minckwitz, G., Jonat, W., Fasching, P., du Bois, A., Kleeberg, U., Luck, H.J., Kettner, E., Hilfrich, J., Eiermann, W., Torode, J. & Schneeweiss, A. (2005). A multicentre phase II study on gefitinib in taxane- and anthracycline-pretreated metastatic breast cancer. *Breast Cancer Res Treat*, Vol.89, No.2, pp. 165-172. ISSN 0167-6806

Ward, H., Chapelais, G., Kuhnle, G.G., Luben, R., Khaw, K.T. & Bingham, S. (2008). Breast cancer risk in relation to urinary and serum biomarkers of phytoestrogen exposure in the European Prospective into Cancer-Norfolk cohort study. *Breast Cancer Res*, Vol.10, No.2, art. no.R32. ISSN 1465-542X

Weller, M., Trepel, M., Grimmel, C., Schabet, M., Bremen, D., Krajewski, S. & Reed, J.C. (1997). Hypericin-induced apoptosis of human malignant glioma cells is light-dependent, independent of bcl-2 expression, and does not require wild-type p53. *Neurol Res*, Vol.19, No.5, pp. 459-470. ISSN 0161-6412

Weyergang, A., Kaalhus, O. & Berg, K. (2008). Photodynamic targeting of EGFR does not predict the treatment outcome in combination with the EGFR tyrosine kinase inhibitor Tyrphostin AG1478. *Photochem Photobiol Sci*, Vol.7, No.9, pp. 1032-1040. ISSN 1474-905X

Wong, C.H., Iskandar, K.B., Yadav, S.K., Hirpara, J.L., Loh, T. & Pervaiz, S. (2010). Simultaneous induction of non-canonical autophagy and apoptosis in cancer cells by ROS-dependent ERK and JNK activation. *PLoS One*, Vol.5, No.4, pp. e9996. ISSN 1932-6203

Xu, L. & Bergan, R.C. (2006). Genistein inhibits matrix metalloproteinase type 2 activation and prostate cancer cell invasion by blocking the transforming growth factor beta-mediated activation of mitogen-activated protein kinase-activated protein kinase 2-27-kDa heat shock protein pathway. *Mol Pharmacol*, Vol.70, No.3, pp. 869-877. ISSN 0026-895X

Xu, L., Ding, Y., Catalona, W.J., Yang, X.J., Anderson, W.F., Jovanovic, B., Wellman, K., Killmer, J., Huang, X., Scheidt, K.A., Montgomery, R.B. & Bergan, R.C. (2009). MEK4 function, genistein treatment, and invasion of human prostate cancer cells. *J Natl Cancer Inst*, Vol.101, No.16, pp. 1141-1155. ISSN 1460-2105

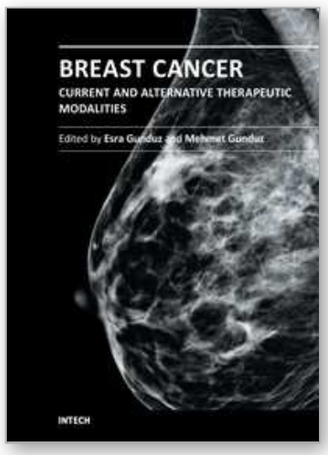
Xue, L., He, J. & Oleinick, N.L. (1999). Promotion of photodynamic therapy-induced apoptosis by stress kinases. *Cell Death Differ*, Vol.6, No.9, pp. 855-864. ISSN 1350-9047

Yeh, T.C., Chiang, P.C., Li, T.K., Hsu, J.L., Lin, C.J., Wang, S.W., Peng, C.Y. & Guh, J.H. (2007). Genistein induces apoptosis in human hepatocellular carcinomas via interaction of endoplasmic reticulum stress and mitochondrial insult. *Biochem Pharmacol*, Vol.73, No.6, pp. 782-792. ISSN 0006-2952

Yoeli-Lerner, M., Yiu, G.K., Rabinovitz, I., Erhardt, P., Jauliac, S. & Toker, A. (2005). Akt blocks breast cancer cell motility and invasion through the transcription factor NFAT. *Mol Cell*, Vol.20, No.4, pp. 539-550. ISSN 1097-2765

Zava, D.T. & Duwe, G. (1997). Estrogenic and antiproliferative properties of genistein and other flavonoids in human breast cancer cells in vitro. *Nutr Cancer*, Vol.27, No.1, pp. 31-40. ISSN 0163-5581

Zhuang, S. & Kochevar, I.E. (2003). Singlet oxygen-induced activation of Akt/protein kinase B is independent of growth factor receptors. *Photochem Photobiol*, Vol.78, No.4, pp.361-371. ISSN 0031-8655



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Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various therapeutic modalities from signaling pathways through various anti-tumor compounds as well as herbal medicine for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

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