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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 cytotoxic 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 demonstrated 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 than 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 μ M (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 ($>10 \mu\text{mol/l}$), while stimulating proliferation of oestrogen receptor positive cells (but not oestrogen receptor negative cells) at lower concentrations ($<10 \mu\text{mol/l}$) (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 phytoestrogen, GE can bind to both oestrogen receptors (ER α /ER β), though it has a higher affinity for ER β than ER α (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 ER α expression in cancerous human breast than in normal breast, while ER β was higher in normal human breast than in cancerous breast. On the other hand, up-regulation of ER β 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 ER α -negative and ER β -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 (enterodiols 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 phytoestrogens (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 (Kulíková 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 ($\cdot\text{OH}$) (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 ($^1\text{O}_2$) (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: $<0.04\ \mu\text{s}$, radius of action: $<0.02\ \mu\text{m}$), 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 relocation 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 photogeneration 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. porphyrinogenic sensitizers and phthalocyanine-related compounds) rapidly mediate $\Delta\Psi\text{m}$ 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 executors 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 (Kulíková 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 $\Delta\Psi_m$ 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 μ M (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 phenotype 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 H₂O₂, which is produced in a variety of tumour cell lines by 1,3-dibutyl-2-thioxoimidazolidine-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 proliferation 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 phosphatidylinositol 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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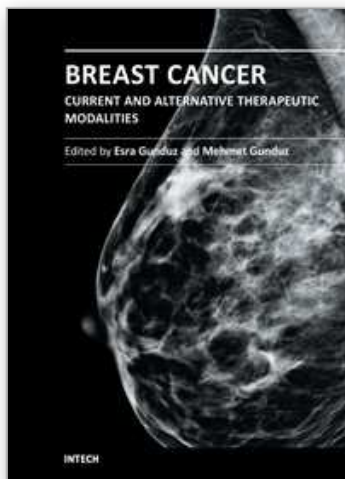
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Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In 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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