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The role of cytoskeleton and adhesion proteins in the resistance to Photodynamic Therapy. Possible therapeutic interventions

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Abstract

It is known that Photodynamic Therapy (PDT) induces changes in the cytoskeleton, the cell shape, and the adhesion properties of tumour cells. In addition, these targets have also been demonstrated to be involved in the development of PDT resistance.

The reversal of PDT resistance by means of manipulating the cell adhesion process to substrata has been out of reach. Even though the existence of cell adhesion-mediated PDT resistance has not been reported so far, it cannot be ruled out.

In addition to its impact on the apoptotic response to photodamage, the cytoskeleton alterations are thought to be associated to the processes of metastasis and invasion after PDT.

In this review, we will address the impact of photodamage on the microfilament and microtubule cytoskeleton components and its regulators in PDT-treated cells as well as on cell adhesion. We will also summarise the impact of PDT on the surviving and resistant cells and their metastatic potential. Possible strategies aimed at taking advantages on the changes induced by PDT on actin, tubulin and cell adhesion proteins by targeting these molecules will be also discussed.

Abbreviations

ALA: 5-aminolevulinic acid; BPD: Benzoporphyrin Derivative; BPD-MA: Benzoporphyrin monoacid ring A; CAM: cell adhesion molecules, CAM-DR: cell adhesion-mediated drug resistance; CAM-RR: cell adhesion-mediated radioresistance; ECM: extracellular matrix, EGFR: epidermal growth factor receptor; FAK: focal adhesion kinase, PDT: photodynamic therapy; ZnPc: zinc(II)-phthalocyanine.

1. Introduction

It is known that Photodynamic Therapy (PDT) induces changes in the cytoskeleton, the cell shape, and the adhesion of tumour cells either directly or mediated by signal transduction processeses. In addition, these targets have been involved in the development of PDT resistance[1].

The three major components of the cytoskeleton –microtubules, microfilaments and intermediate filaments– are known to be affected by PDT. While some authors have found disruption of these three components after PDT[2], actin appears to be one of the most photodamaged cytoskeleton components[3-6] and it is involved in the process of PDT resistance[7,8]. On the other hand, intermediate filaments are slightly damaged[3,9] and microtubules are transiently compromised[10-14] without remaining altered in PDT resistant cells[1,15,16].

The severe morphological changes observed in apoptotic cells suggest that apoptosis has dramatic implications on the cytoskeleton and, as it occurs in chemotherapy, the opposite also holds true for actin during the early stage of photodynamic-driven apoptosis. Likewise in chemotherapy[17-19], cytoskeleton proteins such as actin have been found to play an important role in the early stage of photodynamic-driven apoptosis [20,21].

The mechanism of cell death induced by PDT strongly influences the type of photodamage caused on the cytoskeleton. Ruiz Gonzalez et al.[22] has reported that Porphycene-PDT induces different changes on cytoskeleton components that are different depending on the mechanism of cell death. Besides, PDT through apoptosis causes impairment of the expression of both vinculin and F-actin. Contrarily, PDT through necrosis exerts immediate cell damage thus preventing the disassembly of vinculin from the plasma membrane. Moreover, vinculin proved to have a similar distribution as compared to control cells. However, necrotic photodamage to both microtubules and F-actin was much more severe as compared to apoptotic photodamage.

In addition to their impact on the apoptotic response to photodamage, cytoskeleton alterations are thought to be involved in the processes of invasion and metastasis after PDT [6,7,23], similarly to radio and chemotherapy[24-26].

In this review, we will address the impact of photodamage on the microfilament and microtubule cytoskeleton components and its regulators in PDT-treated cells as well as on cell adhesion. The impact of PDT on the surviving and resistant cells and their metastatic potential will also be summarised. Possible strategies aimed at taking advantages on the changes induced by PDT on actin, tubulin and cell adhesion proteins, by targeting these molecules to overcome drug resistance will be also discussed .

2. Microfilaments

Actin has been identified as the main cytoskeleton component affected by PDT either at early or at late stages of photodamage[3-9,22-23,27-28]. However, the changes induced in the structure of actin filaments seems to depend on the initial morphology of the cell. The 5-aminolevulinic acid (ALA)-PDT has proved to increase the number of stress fibres on human adenocarcinoma WiDr cells, which grow in dense colonies, whereas it caused loss of fibrillar actin structures in growth cones of D54Mg cells, which grow separately[5]. In addition, ALA-PDT of glioma spheroids of human U373 and A172 cell lines exerts a reorganisation of the actin cytoskeleton. A decrease in the amount of fibrillar actin in growth cones of A172 cells was observed, whereas in U373 cells, a rearrangement of actin was found, resulting in a loss of cellular polarisation[23].

Acedo et al[4] have reported that cells subjected to both zinc(II)-phthalocyanine (ZnPc) and the cationic porphyrin meso-tetrakis(4-N-methylpyridyl)porphine-PDT underwent irreversible reorganisation of F-actin upon irradiation, which was accompanied by morphological alterations in cell shape. An extended time course up to 24 h has shown that both focal adhesion kinase (FAK) and F-actin did not return to basal conditions.

We have found that, while long stress fibres were present in the LM3 mammary adenocarcinoma cells, they were shorter or not present at all in ALA-PDT resistant clones derived from LM3. In addition, one of the PDT-resistant clones exhibited a more uneven cortical F-actin layer as compared to the parental line, whereas a waved pattern of cortical actin was observed in the other clone (Figure 1). However, western blot assays revealed that the total amount of β-actin was not modified in the resistant cells[7,29].

We have also found that in normal human mammary HB4a cells, the insertion of an oncogenic form of Ras, induced *per se* disorganisation of F-actin[8]. Ras-transfected cells stained for F-actin revealed the presence of a thick cortical actin rim with an inner fine actin meshwork, and many cells presented short stress fibres mainly organised at the cell periphery and assembled in actin microspikes. These short stress fibres resembled the pattern of migrating cells, where dorsal stress fibres elongate primarily from the leading edge focal adhesions[30]. These features suggest that Ras induces an invasive phenotype.

The distribution of actin on HB4a-Ras cells treated with ALA-PDT, exhibited signals of photodamage, but the original actin pattern was recovered 24 h after treatment. On the other hand, ALA-PDT induced a more dramatic disorganisation of stress fibres in HB4a

cells. Actin microspikes and a perinuclear rim, mimicking non-treated HB4a-Ras cells were present. Surprisingly, 48 h after recovery, some features of actin disorganisation remained present in HB4a surviving cells, resembling Ras-transfected cells. These changes resemble a more invasive phenotype, suggesting that PDT modifies the actin pattern in cells subjected to high PDT doses, and that the cells surviving the therapy exhibit a F-actin distribution phenotype similar to that of invading cells (Figure 2).

The onset of motility necessary for invasion requires a relaxation of static actin structures to form flexible membrane protrusions. Rigid actin stress fibres are disassembled upon the formation of dorsal ruffles, leaving a fine cortical actin meshwork behind, from which cell membrane protrusions like lamellipodia can emerge[31].

However, the pattern detailed above was not found in all the isolated PDT resistant cells Milla et al[15] have analysed actin stress fibres of SCC-13 squamous carcinoma cells that are resistant to methyl-ALA-PDT. F-actin seems to be better organised in the resistant cells as compared to the parental cells, and stress fibres are longer in the most PDT resistant populations.

Since the abnormal expression and polymerisation of actin and the resulting changes to the cytoskeleton are associated with the invasiveness and metastasis of cancers[32], a number of actin-targeting compounds, which disrupt polymerisation dynamics, have been investigated for their capacity to inhibit cell proliferation and motility[33]. Cytochalasins bind to the barbed end of actin filaments, inhibiting monomer association and dissociation, and jasplakinolide induces actin cytoskeleton disruption[34]. However, the universal alteration of actin actually results in high toxicity[35], therefore making difficult its application in clinical trials.

Since F-actin response to PDT is cell-line dependant, and thus PDT resistant cells exhibit either a higher or lower degree of F-actin organisation depending on the cell line and PS employed, it is difficult to predict the outcome of actin-targeted therapies on PDT-surviving cells.

Actin-binding proteins or their upstream regulators can also be targeted: Rho GTPases, filament-depolarising proteins, such as the ADF/cofilin family, gelsolin, and other proteins with actin-filament remodelling activity. The actin scaffolding protein cortactin and actin-binding motor proteins such as myosin II, are also potential targets for cancer interventions. Identification of proteins specifically associated with high degree malignancies is essential to avoid high toxicity in critical organs[35].

GTPases are able to bind a variety of effector proteins and initiate downstream signalling. Rho GTPases regulate important cellular processes ranging from cytoskeletal remodelling and gene expression to cell proliferation and membrane trafficking. RhoA is likely to contribute to the process of cell detachment and the control of rearrangements of the actin cytoskeleton, since it inhibits integrin-based focal adhesions and formation of actin stress fibres leading to cell rounding. Rho proteins also regulate microtubule polymerisation involved in cell migration[36]. RhoE was shown to antagonise RhoA/ROCK signalling and has been implicated in cellular responses involving cytoskeletal rearrangement, including cell migration and invasion. RhoE also affects cell cycle progression and proliferation[37].

Sanovic et al[38] have found an early upregulation of RhoE after Hypericin-PDT in squamous carcinoma cells. In addition, studies of Chang et al[39], suggest that a decreased expression of RhoA was induced after aloe-emodin-PDT of lung carcinoma H460 cells. RhoE was also found to be increased upon exposure of human keratinocytes to UVB to protect them from damage, thus preventing apoptosis. Besides, RhoE was suggested to be a pro-survival factor acting upstream of p38, JNK, p21, and cyclin D1[40]. On the other hand, RhoE antagonises RhoA and inhibits cell cycle progression, in part, by preventing translation of cyclin D1[41].

Kang et al.[42] have suggested the existence of a relationship between RhoA expression and chemoresistance. These authors have found that gastric cancer cells with higher expression of RhoA were more resistant to chemotherapeutic drugs, such as taxol or vincristine, implying that treatment strategies aimed at inactivation of RhoA might be promising to improve the efficacy of these antineoplastics.

Since it is well established that PDT can revert chemoresistance[1], and due to the impact of photodamage on RhoA expression, it is possible that Rho GTPases be involved in the PDT-mediated reversal of drug resistance, though this hypothesis should yet be tested.

3. Microtubules

Evidence that tubulin is a target in PDT was compiled by Berg and Moan in 1997[10]. Microtubules, which are located in the cytosol, have been shown to be depolymerised by PDT treatment[11,43-46]. In addition, several authors found that PDT inhibits polymerisation of microtubules-associated protein (MAP)-free tubulin[10,13].

The co-localisation of hypericin with alpha-tubulin and the aberrant mitotic spindles observed following sublethal PDT doses suggest that photodamage to the microtubule network provokes G2/M phase arrest[47]. In addition, the unpolymerised form of tubulin is a target for PDT, as it was demonstrated employing the microtubule polymerizing inhibitor nocodazole[11]. Lee et al[12] have reproduced the photoinactivation of microtubules *in vitro* with purified microtubule proteins.

However, PDT-resistant cells in general do not differ in the pattern of tubulin expression[1,15,16], thus suggesting that either the mechanism of tubulin photodepolarisation is reversible or that tubulin-damaged cells do not survive the treatment.

In addition, the lack of cell cycle-dependent sensitivity to photodamage in some cases indicates that microtubules may not always be damaged by PDT[10]. Furthermore, when the PDT doses are increased, the importance of microtubule damage in the process of cell inactivation is reduced, because cells die before mitosis [11].

It had previously been thought that actin-myosin played a dominant role in apoptosis-mediated cell remodelling, whereas all other cytoskeletal elements were dismantled. However, the biology of microtubule assembly into the mitotic spindle during mitosis as well as the molecular signalling and the various apoptotic pathways have recently been elucidated[48,49]. It has been proposed that the prolonged mitotic arrest stimulates the apoptotic program, which probably represents a built-in safety mechanism to eliminate cells with deregulated cell cycle components[50].

The microtubule-targeted tubulin polymerising agents, such as paclitaxel and taxotere, inhibit microtubule dynamic instability, cell cycle G2/M phase transition and mitotic arrest of cancer cells; and these effects trigger the molecular signalling for the mitochondrial pathway of apoptosis[48].

Additive effects of PDT in combination with paclitaxel in cell lines of different origins have been demonstrated[51,52]. Indeed, no cross-resistance has been found for these treatments[53]. Since monomeric tubulin is more sensitive to PDT than polymerised tubulin, Ma et al have administered microtubule inhibitors to tumour-bearing mice prior to light exposure. These authors have found that the microtubule inhibitor vincristine enhanced PDT employing a sulphonated tetraphenyl porphine as a photosensitiser, indicating that the antineoplastic and PDT may act synergistically on microtubules function[54].

It has been reported that Photofrin-PDT partially reverts the highly resistant Friend leukaemia cell line ADM-RFLC to antitubulin drugs such as vinblastine or vincristine[55]. We have also found that taxol can revert resistance in our mammary adenocarcinoma ALA-PDT resistant clones (unpublished results).

Due to the photosensitivity of unpolymerised tubulin, the experimental data reviewed herein, suggest that it is possible to employ PDT in combination with anti-microtubule agents, since these drugs have probed capable of reversing PDT resistance.

4. E-cadherin

E-cadherin, the prototypical member of the classic cadherin family, mediates cell-cell adhesion in epithelia. For cadherin functioning, the cytoplasmic domain of these proteins must bind to the actin cytoskeleton via proteins named catenins[56]. Loss of E-cadherin expression leads to epithelial tumorigenesis[57].

The deregulation of E-cadherin adhesion is a crucial step during cell migration and metastasis and, therefore, many epithelial cancer cells can dynamically repress E-cadherin expression to initiate the migration process[58].

The treatment of Pam212 keratinocytes with ZnPc and light induces a rapid dismantling of E-cadherin complexes, followed by the activation of a cell death program, a mechanism that resembles the action of E-cadherin blocking antibodies[59].

Alterations in the distribution but not in the expression of E-cadherin were observed in our LM3 mammary adenocarcinoma ALA-PDT resistant clones. These features were particularly marked in one of the two clones isolated, where E-cadherin was distributed as interdigitations, which account for a higher cell-cell cohesion and a higher differentiation level[7] (Figure 1). Since it has been suggested that cell adhesion to collagen I is regulated by the functional state of E-cadherin[60], we ascribed the more marked binding of the resistant clones to collagen I to a higher E-cadherin disorganisation. However, other authors have not found similar changes of E-cadherin distribution in methyl-ALA-PDT-resistant cells isolated from the SCC-13 squamous carcinoma cell line[15].

The expression of E-cadherin is downregulated during tumour invasion and metastasis[61,62]. An inactivation of the E-cadherin encoding gene, CDH1, is frequently observed in tumours. The latter fact implies an increase in the ability of the tumour to

survive and invade other tissues[63,64]. A downregulation of this gene is commonly observed in epithelial tumours and is a hallmark of the epithelial to mesenchymal transition. Due to its rapid inactivation, it is difficult to target the E-cadherin protein directly as an anti-cancer strategy. However, the loss of E-cadherin creates vulnerabilities in the tumour cell that could then be targeted with drugs. A search for proteins which, if inactivated, lead to the death of cancer cells lacking E-cadherin, but not cells with normal levels of E-cadherin, is currently being carried out[65].

Taking into account the results of Espada et al [59] which indicate the photosensitivity of E-cadherin complexes, the combination of PDT with therapies aimed at targeting disrupted E-cadherin is worth investigating.

5. Integrins and cell adhesion

The inhibition of cell adhesion to collagen, fibronectin, laminin and vitronectin after PDT with Benzoporphyrin Derivative (BPD) has been described. When the cell type exposed to PDT was OVCAR 3 ovarian carcinoma, the loss in adhesiveness was accompanied by a loss of β1 integrin-containing focal adhesion plaques[66], whereas the PDT performed on human foreskin fibroblasts did not induce any change in integrin expression, but decreased FAK phosphorylation, this phenomenon being probably related to integrin signalling[67]. These findings suggest that the cell type is critical for the integrin response to PDT. Uzdensky et al[68] have shown that sub-lethal ALA-PDT inhibited cell adhesion as well, together with redistribution of integrins and formation of new focal adhesions. In addition, as mentioned above, our ALA-PDT resistant LM3 mammary carcinoma clones, exhibited higher binding to collagen I, as compared to the parental lines, without overexpressing β1 integrin[29].

Often, adhesion studies performed immediately after PDT demonstrate a decreased adhesion either to ECM or to other cell types, but this pattern appears to be recovered at long periods of time after PDT[66]. PDT-surviving cells do not show a consistent pattern of ECM cell adhesion modifications. On the other hand, adhesion to plastic and consequently, resistance to trypsinisation seem to be factors usually affected after PDT, and cells surviving to photodamage are usually more adherent to plastic[1,16,68].

Integrins are obligate heterodimeric transmembrane receptors consisting of one alpha and one beta subunit, which function as adhesion molecules for ECM proteins[69]. Due to their

expanded network of interaction partners including adapter and signalling molecules, integrins not only connect the cell cytoskeleton with the extracellular microenvironment but also they control critical cell functions such as survival and migration through the activation of certain signalling mechanisms[70,71].

It has been hypothesised that integrins are a target in PDT-induced impairment of cell attachment and apoptosis. It has been described that after Hypericin-PDT, the genes encoding integrins ß1, α3 and α6 were downregulated at 1 to 3 h after treatment, leading to a reduction in signal transduction from ECM as well as to an impaired cell adhesion in the early phase of photodamage. The latter conditions are known to be required for cell cycle arrest and apoptosis[38]. However, at 8 h post PDT, the integrin encoding genes tend to recover their initial expression levels.

Galaz et al[72] have found a more sustained impairment of ß1 integrin expression until 48 h post PDT with zinc (II)-phthalocyanine (ZnPc). The expression level of β1 integrin remained at basal levels for at least 18 h in Pam212 keratinocytes. At this timepoint, the expression of β1-integrin decreased sharply, a phenomenon that was coincident with the detachment of cells from the substratum and which was sustained for at least until 48 h after photodamage. Impaired mRNA expression of ß1 integrin was also observed in the human laryngeal cancer HEp-2 cells 12 h following PDT with aluminium phthalocyanine tetrasulfonated (AlPcS4) and ZnPc as photosensitisers[73]. In addition, downregulation of thrombospondin-1, a ß1 integrin ligand, was also observed in Hypericin-PDT treated A-431 cells[38].

Non-lethal ALA-PDT has been shown to inhibit the attachment of colon carcinoma WiDr cells to the plastic substratum, to impair the trypsin-induced cell detachment from the plastic surfaces, and to induce a redistribution of $\alpha_v\beta3$ integrin[68,75]. These authors have suggested that PDT-induced inhibition of trypsin detachment may be due to integrin cross-linking within the pre-existing adhesion clusters and cross-linking of integrins with other proteins in the focal contacts.

The adhesive protein fibronectin and its integrin receptors play an important role in tumour development and mediate ECM function. A decreased fibronectin expression had been correlated with tumorigenic phenotypes[76,77]. However, more recently, an increased fibronectin expression has been related to poor prognosis in ovarian and breast carcinomas[78-80].

Ruhdorfer et al[74] have observed a dramatic downregulation of the fibronectin gene after ALA-PDT of the squamous cell carcinoma line A-431. This suppression was the

strongest when compared to the other genes that were modified and occurred at all timepoints tested.

The generation of tumour-associated fibronectin isoforms allows the development of specific ligands, which can be used for the selective delivery of therapeutic agents to the tumour environment. Thus, fibronectin is being used as a target for biomolecular intervention, both for the development of inhibitory molecules blocking the interaction of fibronectin with integrins on the cell surface, and ligand-based therapeutic strategies[81]. However, there is not enough evidence to speculate about possible interactions between PDT and fibronectin interventions.

6. Cell adhesion-mediated drug resistance and radioresistance

Adhesion is a hallmark of haematological and solid cancer cells. All five classes of cell adhesion molecules (CAM) –integrins, cadherins, immunoglobulin-like CAMs, selectins and CD44s– are characteristically deregulated in human cancer. Adhesion enables and promotes cancer-defining biological processes such as growth, survival, migration, extravasation, homing, and metastasis. Recently, several cell adhesion-mediated survival pathways have been elucidated, with key mediators being LFA-1, VLA-4, FAK, ILK, Src, PI3K, Akt, Ras, MEK, Erk, HMG-CoA reductase, Rho, Rho inase, PKC, and NFkB[82].

The onset of drug-resistance to chemotherapy phenotypes is often associated with altered expression of adhesion and cytoskeletal components. Among the myriad of microenvironmental factors impacting on cancer cell resistance, cell adhesion to the ECM has recently been identified as a key determinant. Experimental evidence shows that anti-apoptotic pathways initiated by cell adhesion are operative in tumour cells and, furthermore, they cause resistance to mechanistically distinct cytotoxics. The phenomenon has been termed cell adhesion-mediated drug resistance (CAM-DR) and is based on the observation that cells that adhere to ECM components are protected from apoptosis induced by chemotherapeutic agents[83].

We were interested in assessing if cell-adhesion-mediated PDT resistance was likely to occur. Based on the observation that the two LM3 ALA-PDT-resistant clones isolated in our laboratory presented a higher spreading than the parental line (Figure 1), we tested the hypothesis that ALA-PDT could revert resistance in cells grown in suspension. We

have found that both for the parental and the resistant clones, the light doses necessary to induce cell killing were half the doses necessary to kill the cells growing attached to the plastic. However, no differences in the resistance indexes were found. We have also performed ALA-PDT in photoresistant cells attached to fibronectin, to see if the cell adhesion to its ECM proteins could revert PDT resistance, but again, we did not find differences in the resistance indexes of the clones[84]. We also intended to revert the intrinsic resistance to PDT of human mammary HB4 cells transfected with an oncogenic form of Ras, by applying ALA-PDT to a cell suspension. Again, reversion of resistance was not obtained[85]. However, the existence of cell adhesion-mediated PDT resistance mechanisms not yet reported cannot be ruled out.

Integrin-associated signalling is highly involved in the process of CAM-DR. In addition to their structural functions, integrins mediate signalling from the extracellular space into the cell through integrin-associated signalling and adaptor molecules. A huge number of clinical trials based on integrin inhibition for treatment of tumours have already been completed while others are under way. Taking into account the diversity in expression and activation of integrin- and growth factor receptor-associated signalling molecules, a better understanding of the molecular differences between cancer cells and normal cells is likely to promote the development of therapies that specifically target cancer cells, including antibodies, interference RNA or small molecule inhibitors[86]. These integrin-targeted therapies could be potentially combined with PDT to revert chemoresistance.

In addition to a mechanism of chemotherapy resistance associated to adhesion, a similar mechanism of radiotherapy resistance has been described[87]. Despite the differentiation between CAM-DR and cell adhesion-mediated radioresistance (CAM-RR), the underlying mechanisms share features related to integrin and focal adhesion hub signalling and differ further downstream in the complexity of signalling networks between tumour entities[71].

Among a plethora of putative therapeutic strategies aimed at overcoming CAM-RR, a number of studies have focused on the concept of tightly connected epidermal growth factor receptor (EGFR) and integrin signalling pathways[71,88]. It has been found that integrin-mediated adhesion to ECM induced EGFR phosphorylation, which could be prevented by silencing β1 integrin[89,90]. β1 integrin is an essential regulator of EGFR signalling and tumorigenic properties of some cancer types, and its silencing might represent an adjuvant approach to anti-EGFR therapy. Treatment of patients with human

squamous cell carcinomas of the head and neck with the humanised anti-EGFR antibody C225 in combination with fractionated radiotherapy resulted in improved locoregional tumour control and overall survival[91]. Dual targeting of EGFR and β1 integrin enhanced the efficacy of EGFR inhibition on radiation survival of head and neck squamous cell carcinomas[92]. A recent analysis also showed that colocalization of EGFR and β1 integrin in biopsies of high grade astrocytomas correlated with prognosis and outcome[93]. These findings strongly underscore the concept of mutual and cooperative integrin–EGFR interactions in the context of CAM-RR[71].

Del Carmen et al[94] have tested a combination schedule consisting of C225, and BPD-MA-PDT in a mouse model of human ovarian cancer, and they observed a synergistic effect of C225 and PDT. Such synergism could be ascribed either to the enhancement of EGFR phosphorylation driven by PDT or to an increase in the activity of downstream signalling members, such as ERK 1 and ERK 2 induced by PDT[95,96], which are also regulated by integrins signalling pathways[97]. One possible hypothesis for the synergistic action of PDT and therapies aimed at inhibiting EGFR is that it might be mediated by PDT-induced impairment of integrins expression.

7. Metastasis

Any alterations in the three major components of cytoskeleton have been related to tumour progression and metastasis. In view of the *in vitro* evidence detailed above PDT-treated cells show a pattern of decreased integrin and fibronectin expression. These features are usually correlated with decreased metastatic dissemination [78-80,86]. On the other hand, a transient inhibition of adhesion to ECM and an actin distribution pattern resembling the pattern of invading cells are observed on some PDT surviving cells. According to these features, metastatic dissemination of PDT-surviving cells would be expected to increase.

Evidences suggest that cells surviving PDT have a decreased metastatic *in vivo* potential[23,29,98-102]. On the other hand, Momma et al[103] have reported an increased impact on the metastasic capacity of PDT-surviving cells after sublethal BPD-MA-PDT of prostate cancer MatLyLu cells.

However, the metastatic capacity of *in vitro* PDT-treated cells when they are injected into mice could be quite different from the metastatic rate of an implanted tumour after

in vivo PDT treatment. In this regard, the impact of PDT on the endothelial barrier becomes important. Chen et al[6] have carried out vascular-targeting PDT employing Verteporfin, and have found that the vascular barrier permeability was increased in prostate MatLyLu tumours injected to mice, probably due to endothelial cells retraction, leading to the formation of intercellular gaps, which could eventually have an impact on the capacity of tumour cells to extravasate and metastasise.

In vitro, a decreased invasive and migratory phenotype of PDT-treated cells has been reported for a wide range of photosensitisers[16,23,27,101,104-108]. On the other hand, the migration of mesenchymal stem cells towards glioblastoma cells was enhanced after ALA-PDT treatment[109].

HPD-PDT decreased adhesiveness of cancer cells to endothelial cells *in vitro*, has been found to correlate with a decreased metastatic potential of HPD-PDT treated cells injected into rats[101,104,110]. This impaired metastatic rate was not related neither to a lower tumour take nor to a decreased tumour size, but it was correlated to a transient decrease in the expression of adhesion molecules[110].

Figure 3 summarises the different steps where PDT may impact on the metastasis cascade.

Our research group has found that ALA-PDT-adenocarcinoma resistant cells are less invasive and tend to migrate less *in vitro*, while *in vivo* they induce less metastasis foci as compared to the parental cell line. In addition, they showed a lower tumour take, latency time and growth rate and anchorage-dependent adhesion[29].

To sum up, further work is needed to unveil the crosstalk between pro- and anti-metastatic signalling in PDT surviving cells.

8. **Conclusions and future directions**

An increasing bulk of evidence suggests that PDT induces changes on the actin and tubulin cytoskeleton, and on cell-cell adhesion and adhesion to substrata, summarised in Figure 4. However, the type of photodamage and the features of PDT-surviving cells seem to depend on the cell type, photosensitiser and light dose. Employing low PDT doses, surviving cells might be able either to maintain the photodamaged features or to recover their initial patterns of molecules expression. Alternatively, if the PDT dose is high, the majority of cells might die, thus selecting non-impacted cells which will

survive and duplicate, leaving PDT resistant populations with similar features as compared to the non-treated cells.

Further work is needed to elucidate the mechanisms of PDT resistance and to develop treatments aimed at reverting chemoresistance by photodamaging cytoskeleton and cell adhesion proteins.

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Figure legends

Figure 1: Features of ALAPDT resistant clones derived from LM3 carcinoma cells

ALA PDT resistant clones 4 and 8 exhibited altered E-cadherin and F-actin expression, and higher spreading, whereas the microtubule structure remained unchanged.

Resistant clones were found to be less metastatic than their parental counterparts and less invasive *in vitro*. Adapted from Casas et al 2006[84], Casas et al 2008[7] and Casas et al 2008[29]. We acknowledge Elsevier for having given permission to reproduce information contained in Figure 1, which was previously published in [7] and [29] and to Spandidos Publications to reproduce material published in [84].

Figure 2: F-actin distribution on HB4a and HB4a-Ras cells surviving PDT

In normal human mammary HB4a cells, insertion of an oncogenic form of Ras, induced *per se* disorganization of F-actin. The presence of short stress fibres mainly organised at the cell periphery resembles the pattern of migrating cells, suggesting that Ras induces an invasive phenotype. HB4a cells recovered after ALA PDT resemble actin features of

Ras-transfected cells. Adapted from Di Venosa et al 2012[8]. We acknowledge Elsevier and Spandidos Publications for having given permission to reproduce information contained in Figure 2, which was previously published in [8].

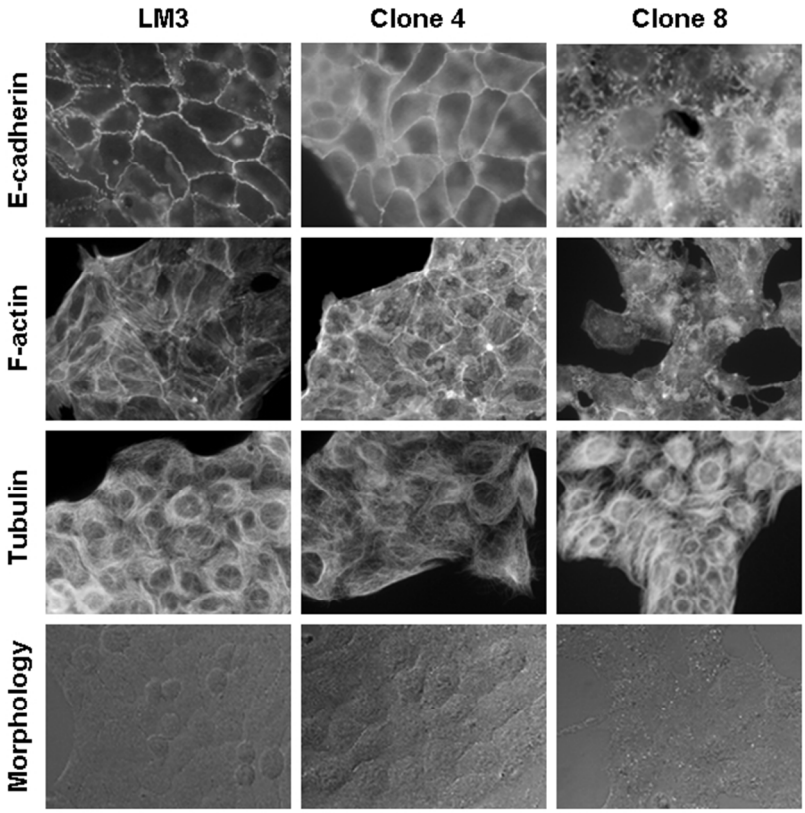
Figure 3: Impact of Photodynamic therapy on major steps of the metastasis cascade

PDT targets both tumour-tumour and endothelial-tumour cell adhesion, thus affecting detachment from primary tumour, attachment to the target organ, as well as intra- and extravasation. In addition, PDT can affect endothelial cells causing retraction, thus favouring tumour cell intra- or extravasation. Migration of detached cells is also influenced by PDT treatment. In addition, molecular targets of PDT such as cadherins, integrins, tubulins, actin, and fibronectin among others, may be photodamaged, thus impacting on the metastasis outcome.

Figure 4: Changes induced by PDT on cytoskeleton and molecules involved in cell adhesion. Possible interactions of PDT with molecular targeted therapies

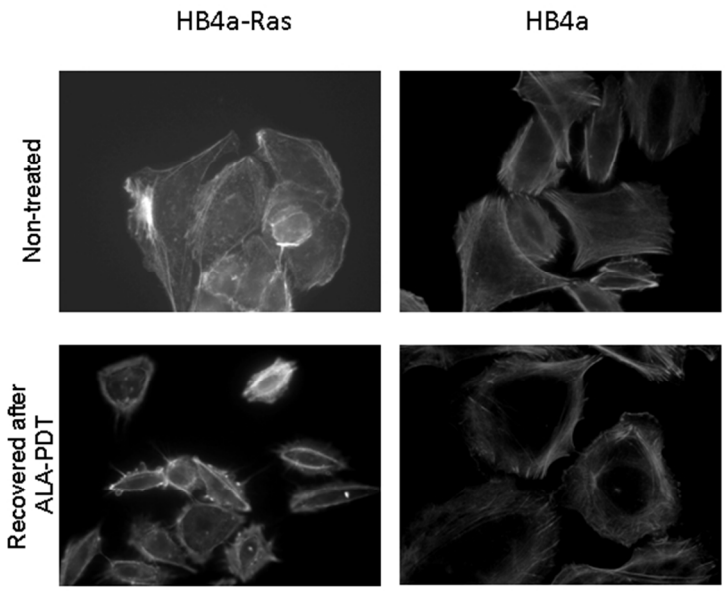
PDT, through apoptosis or necrosis, impacts on cytoskeleton elements and adhesion molecules as well as on their regulatory molecules. These changes influence cell adhesion to substrata. However, relatively few of these features remain present in PDT-surviving cells, and this is probably related to the PDT dose employed, the selection method. Studies aimed at describing cytoskeleton and adhesion of PDT resistant cells are scarce.

Arrowheads indicate possible ongoing or experimental molecular targeted therapies which could potentially interact with PDT, enhancing its effect or reverting chemoresistance.

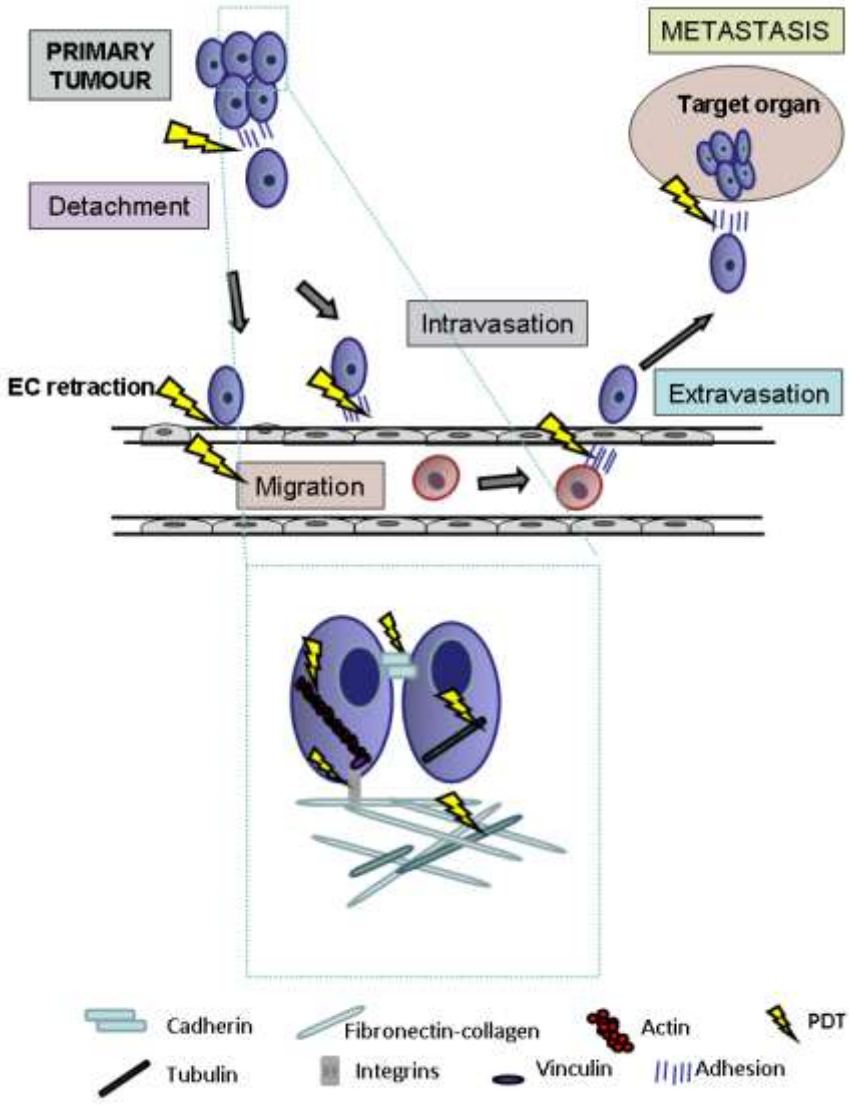


	LM3	Clone 4	Clone 8
Tumour take	+++	++	+
Metastatic potential	++++	+	-
Invasiveness	++++	+	+
Plating efficiency	+	+	+

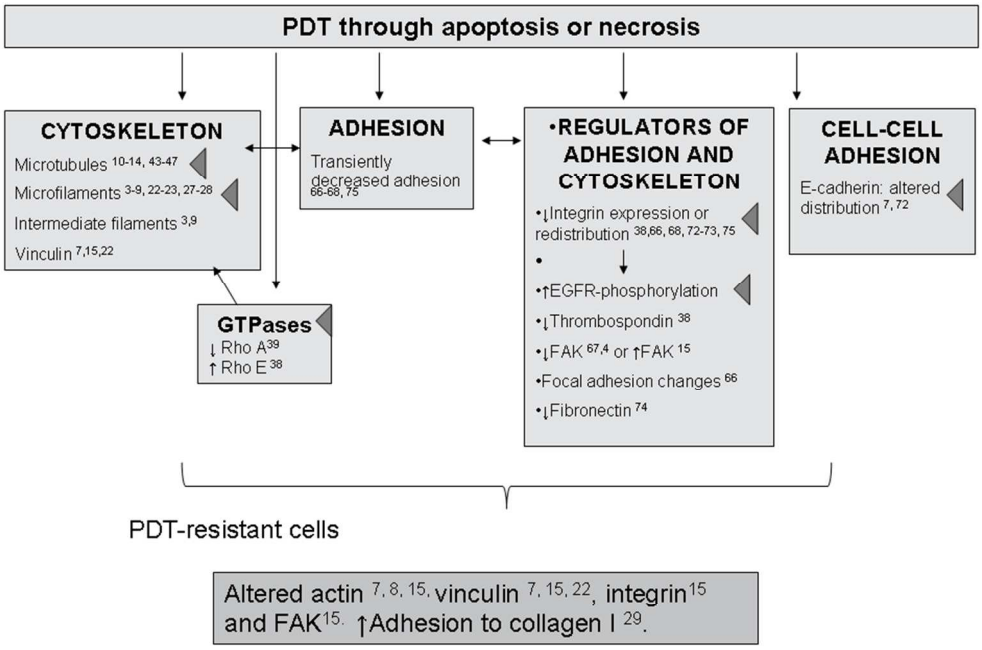
190x254mm (96 x 96 DPI)



190x254mm (96 x 96 DPI)



190x254mm (96 x 96 DPI)



254x190mm (96 x 96 DPI)