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Review

Enhancing photodynamic therapy in skin cancer: important considerations to increase PDT efficacy

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ABSTRACT

Despite significant scientific advances over the last decade in the field of chemotherapeutics and cellular targets, there still remains the need for improved therapeutic modalities. Photodynamic therapy (PDT), a minimally invasive therapeutic modality, has been shown to be effective in a number of oncologic and non-oncologic conditions. However, in the skin cancer milieu, a number of factors contribute to therapeutic resistance. Two important considerations include the tumor microenvironment (TME) and the expression and/ or upregulation of ATP-binding cassette (ABC) transporters. The TME is hypoxic and has a low pH. Furthermore, it comprises the extracellular matrix (ECM) proteins (e.g. Matrix metalloproteinases and collagens), adhesion molecules (eg. integrins and cadherins), cancer-associated fibroblasts, endothelial cells, cytokines and immune cells (neutrophils and macrophages). PDT has been shown to affect ECM proteins such as MMPs and collagen as well as the expression of certain adhesion molecules such as intracellular adhesion molecule 1 (ICAM-1) and vascular adhesion molecule 1 (VCAM-1). Furthermore, PDT seems to increase cytokine secretion of IL 6 and tumor necrosis factor α (TNF-α) and vascular endothelial growth factor, thereby directly stimulating the immune response and the vascular system. In addition, we and others have recently reported on the efficacy of PDT in combating melanoma due to photosensitizer co-localisation within melanosomes. This data was in contrast to reports where the overexpression of transporters ABCC1 and ABCG2 in adenocarcinoma cancer reduced the hypericin-mediated PDT killing efficiency. PDT resistance has also been recently linked to a small subpopulation of cancer-associated stem-like cells which survive through autophagic induction. This review will explore the effect of PDT on both the TME and the family of ATP transporters and attempt to elucidate mechanisms which potentially will enhance the destruction of these recalcitrant tumors.

KEYWORDS: photodynamic therapy (PDT), skin cancer, tumor micro-environment (TME), ABC transporters

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INTRODUCTION

Skin cancer is the third most frequent malignancy in the world. Clinically presenting as non-melanoma and melanoma skin cancer [1], non-melanoma skin cancer (NMSC) is the most common skin cancer type, accounting for 95% of all skin cancers. NMSC is deemed the most common tumor-type worldwide, with an estimated 2-3 million cases occurring per year [2, 3]. Although NMSC is rarely fatal, it often results in aesthetic disfigurement, especially on the head, face and neck [2]. It can present as either basal cell carcinoma (BCC) or squamous cell carcinoma (SCC) which stem from mutations induced in keratinocytes. In contrast, melanomas arise from mutations induced in melanocytes [4-6]. Eighty percent of skin cancers are diagnosed as BCC.

These cancers are characterized as slow growing and rarely metastatic with mutations in the patched (*PTCH1*) and *TP53* tumour suppressor genes [4, 5, 7]. SCC, in contrast, is more aggressive and less frequent (1:4) than BCC [4]. SCC occurs as a result of mutations in the *TP53*, $P16^{INK2A}$ genes, *AP1* transcription factor complex and the pro-inflammatory cyclooxygenase (COX2) [2, 4]. In melanocytes, the most common genetic mutations which give rise to melanoma are mutations in the *BRAF*, *N-RAS*, *CDKN2A* and *PTEN* genes [6, 8, 9]. Other less common mutations which result in a carcinogenic phenotype include MEK, ERK, ARF, P53, AKT and MITF [6, 8]. Melanoma, despite only accounting for 5% of all skin cancers, has the greatest potential to metastasize and is responsible for approximately 85% of skin cancer-related deaths [6, 9]. Shockingly, the prognosis for an estimated 14% of patients with metastatic melanoma is 5 years or less [6]. Moreover, the World Health Organization (WHO) has estimated that melanoma has the fastest growing incidence rate compared to all cancers: doubling every 10-20 years [10].

Skin cancer treatment

Once a NMSC or a melanoma is diagnosed, the current golden standard of treatment is surgical resection [11-13] followed by bouts of adjuvant therapy including chemotherapy or ionizing radiation [9]. However, melanoma is often resistant to these traditional therapies which is reflected by its high recurrence rate, poor patient prognosis and multi-drug resistance [9, 14, 15]. A need therefore exists for novel adjuvant melanoma therapies [15]. Photodynamic therapy (PDT) is a promising potential therapy for melanoma [15, 16]. Furthermore, it is an effective treatment modality offered clinically in some parts of Europe and the USA for the treatment of NMSC, especially in NMSC that occurs on sensitive areas of the face, head and neck which are difficult to resect [12, 13, 17]. PDT is a minimally invasive therapy that results in good cosmetic outcome which can also be applied topically to cancerous skin lesions [12, 13, 17-19]. The efficacy of PDT relates to light activation at a specific wavelength of a photosensitizer (PS), in the presence of molecular oxygen [20]. In addition, it depends on the intracellular accumulation of the PS within the tumour and availability of molecular oxygen to cause effective tumour destruction [19-21]. However, even being an effective emerging therapeutic modality, resistance to PDT has still emerged in various cancers. Besides highlighting cancers' heterogeneity, this characteristic has also been suggested to be related to several resistant mechanisms such as defective apoptotic pathways, over-expression of antioxidants and accumulation of the PS within the tumour to a level below its cytotoxic threshold [20, 22, 23]. Due to this, a number of considerations in the therapeutic approach need to be adopted. Two recent considerations include the tumor microenvironment (TME) and the expression and/or upregulation of ATP-binding cassette (ABC) transporters.

PDT and the tumor microenvironment (TME)

The tumor microenvironment (TME) surrounds a skin cancer *in vivo* and through bi-directional communication between the skin tumor and its TME, angiogenesis, tumor proliferation, metastasis and tumor resistance can be initiated and controlled [24, 25]. The stroma of the TME has a stiff architecture and consists of ECM proteins (collagen, elastin, laminin, vibronectin and fibronectin), which make the skin cancer rigid, transformed fibroblasts and immune cells such as macrophages and T-lymphocytes [24-27]. It is known that the tumor-associated stroma is a crucial part of the tumor microenvironment and can contribute to cancer proliferation, invasion and metastasis [24, 27, 28]. As the stroma consists of extracellular matrix (ECM) proteins and cancer-associated fibroblasts (CAFs); it is particularly important to investigate the effects of PDT on ECM proteins and CAFs, particularly their role in cancer cell resistance to PDT [24, 26-32] CAFs deposit ECM proteins such as collagen that contributes to the stromal milieu of proteins, attributing to the stiff architecture of a tumor [26, 27]. Tumor stiffness from abnormal, hyper ECM deposition is thought to be one of the key factors that influences cancer cell invasiveness, motility and invasion and has been associated with poor patient prognosis, thereby suggesting its possible role in tumor resistance to therapies [26, 28, 29, 33].

Matrix metalloproteinases (MMPs)

Another important family of proteins in tumor-associated stroma are matrix metalloproteinases (MMPs). MMPs occur as several types (1, 2, 4, 6 and 9) and their enzyme activities are dependent on their substrate [34, 35]. Examples of this include MMP2 and 9 which can both deposit and degrade collagen [26, 36, 37]. This therefore produces a biological conundrum - if PDT induces the expression of MMPs that degrade collagen, the therapy would be considered favourable and contributes to PDT efficacy as the TME is being targeted and eradicated. However, this degradation of collagen in the TME by MMPs has also been suggested to enhance skin tumor metastasis, invasion and angiogenesis and thus, be rendering PDT less efficacious [36, 37]. For example, PDT using the photosensitiser hypericin decreased expression of MMP 9 in nasopharyngeal cells which was suggested to prevent metastasis and angiogenesis as MMP 9 has been shown to contribute to tumor angiogenesis and growth [38, 39]. However, after topical PDT of the skin using the photosensitiser MAL, an increased expression in MMP 1 and 9 was associated with improved healing and skin aesthetic post-treatment [40]. Furthermore, PDT in combination with matrix metalloproteinase inhibitors resulted in an improved PDT efficacy in a mammary carcinoma model *in vivo* [38]. Therefore, should PDT induce MMPs that deposit collagen this may further contribute to skin tumor resilience due to stroma stiffness of the TME which may also infer resistance to treatment but this may also improve healing of the lesion and halt skin cancer metastasis, invasion and angiogenesis. Additionally, should PDT enhance MMP expression which results in collagen degradation, it may contribute to tumor invasiveness, metastasis, and angiogenesis thereby encouraging skin tumor growth and metastases.

This dichotomous concept promotes the need to investigate the effects of PDT on MMP expression [38, 41-43]. Ferrario *et al*. (2004) showed that PDT using the photosensitiser porfimer sodium enhanced MMP 2 and MMP 9 activity in a murine mammary tumor model [38]. Additionally, PDT of the skin condition scleroderma, using aminolevunilic acid (ALA)-PDT, displayed a singlet oxygen-dependent increase in MMP 1 and MMP 9 and a decrease in mRNA expression of collagen 1 in normal fibroblasts [43]. PDT can also result in MMP production by fibroblasts via a paracrine mechanism [42, 44]. It has been shown that this mechanism can be induced by soluble factors which are released by keratinocytes that were treated with ALA-PDT [42, 44]. More specifically, a 3-fold increase in collagen-degrading MMP1 and MMP3 but no change in collagen I was observed in fibroblasts that were treated with conditioned medium from ALA-PDT treated keratinocytes [42]. This showed therefore that the type of MMP induced via PDT varies, and is dependent on the photosensitiser used during the treatment regime. Interestingly, it has not yet been investigated whether the same photosensitiser induces similar or different MMP expression by CAFs of different cancer types. This could be an interesting avenue to explore to further elucidate resistant mechanisms to PDT.

Integrins and cadherins

Another important group of ECM proteins that reside in the skin cancer TME are adhesion molecules (integrins and cadherins) which mediate skin cancer metastasis from the primary tumor site, cell migration through the TME and invasion into blood vessels in the dermis of the skin [45-48]. To date, only a few studies have investigated the effects of PDT on integrins and cadherins. Summarily, these studies suggested that different photosensitisers induce different adhesion molecule expressions which seem to be cancer cell type-dependent [30]. For example, PDT treatment of ovarian carcinoma cells using the photosensitiser, benzoporphyrin derivative (BPD), resulted in β-integrin decrease [49] while PDT treatment using 5-ALA on adenocarcinoma (WiDr) cells caused $\alpha_V\beta_3$ redistribution of expression but no change in E-cadherin was observed when WiDr cells were cultured in suspension pre-treatment [50]. Furthermore, post treatment with ALA-PDT of squamous cell carcinoma cell lines (SCC-13) was shown to lead to an increase of β1-integrins [51]. Since squamous cell carcinoma and melanoma are both potentially metastatic skin cancers, further studies are needed using different photosensitisers to explore the effects of PDT on adhesion molecules.

In vitro 3-D models

The recent trend in targeting the TME during PDT when conducting *in vitro* studies involves 3D *in vitro* models comprising cultured cells on an ECM-like layer which may also include immune cells, endothelial cells and fibroblasts. Growth-factor reduced matrigel (BD Biosciences) was used in several studies as an ECM component of the TME, when investigating PDT of various cancers and using different photosensitisers [52-55]. Glidden *et al*. (2012) [53] cultured pancreatic cancer cell lines (APC-1 and PANC-1) on top of a Matrigel™ layer and observed spontaneous nodule formation which mimicked nodule formation of pancreatic tumors *in vivo*. They then used this 3D model to investigate PDT parameters using the photosensitiser benzoporporphyrin derivative monoacid ring-A and found unsurprisingly that PDT parameters in a 3D *in vitro* model are more complex to account for, than in a 2D i*n vitro* model [53]. This complexity was further emphasised in a study which used the photosensitiser EtNBS for PDT on ovarian cancer cells suspended in Matrigel™ [52]. The ECM component allows for the development of nodules which have a hypoxic core which is a key characteristic of a tumor's hypoxic centre and is often resistant to therapies [52, 53]. These ovarian cancer nodules were also coated with the ECM proteins fibronectin, collagen IV and laminin V [49]. Once they had developed this model, the same group further investigated the effects of BPD on a 3D ovarian cancer model which used OVCAR5 cell suspension seeded on Matrigel™ and showed that ovarian cancer cells were twice as resistant to a synergistic PDT and chemotherapy treatment in a 3D TME when seeded on the Matrigel™ [56] compared to a 2D monolayer culture [54, 55]. These studies suggested that 3D *in vitro* models using Matrigel™ as an ECM substitute improve recapitulation of critical characteristics of tumor biology and may have the potential to bridge the gap between labour intensive and expensive animal studies and preliminary *in vitro* studies, when investigating tumor response to therapy [54, 55].

Moreover, the ECM component can be included in *in vitro* 3D models using other ECM proteins such as collagen or fibrin and synthetic scaffolds. As fibroblasts are also a key component of the TME, several studies have investigated the effects of PDT on fibroblasts and the ECM [26, 56, 57]. One study showed a decrease in TGFβ and bFGF in fibroblasts when a collagen matrix was treated with PDT [58]. A collagen matrix made up of collagen such as type I rat tail collagen or fibrin can also be used as an ECM component in 3D *in vitro* studies [56, 59]. Another recent study by Alemany-Ribes *et al*. (2013) [57] used a synthetic scaffold consisting of a self-assembling hydrogel that comprises RAD-16 peptide molecules (BM™PuraMatrix™) and cultured normal primary human fibroblasts seeded on an insert on top of the hydrogel. The photosensitiser uptake by cells buried in the hydrogel construct was ineffective [57]. This result interestingly suggests that CAF may not take up photosensitisers effectively, especially as they are buried deep in the stroma of the TME. Another perspective to this may be related to the differentiation state of the CAFs if indeed they are adopting an "undifferentiated, stem cell-like state" due to the influence of the TME. This result re-emphasises the fact that investigating the effects of PDT on cells in classic 2D monolayer *in vitro* systems may not be sufficient to yield enough evidence on the efficacy of PDT and that the future trend should be an encouragement of 3D model use.

To the best of our knowledge, 3D *in vitro* models using an ECM component and skin cancer cells (melanoma, basal and squamous cell carcinoma) have not yet been investigated for their response to PDT. However, there are some 3D *in vitro* skin models which have been reviewed comprehensively [60]. Examples include cell free matrices, de-epidermized epidermis scaffolds, inert filters, synthetic scaffolds, ECM protein coated membranes, inert filters, collagen and fibroblast collagen hydrogels and commercially available skin models [60]. However, none of these 3D *in vitro* skin models have been used in a PDT setting. It is not surprising that 3D *in vitro* cultures are fast becoming the preferred *in vitro* system when investigating PDT efficacy for the treatment of cancers because they allow for improved cell interaction with the ECM of the TME. Our laboratory is currently developing an *in vitro* 3D skin cancer model which will further be used to investigate the effects of PDT using the photosensitiser hypericin.

Overcoming photodynamic therapy resistance by targeting ABC transporters

The second consideration relating to therapeutic resistance can be attributed to a family of proteins called ATP-binding cassette (ABC) transporters, which drive efflux of various cytotoxic anticancer drugs, photosensitizers, chemotherapeutic and other cellular noxious agents and thus leads to treatment failure [61-65]. In humans, ABC transporters possess 48 genes, which are ubiquitously expressed and located on the cell membrane and multiple subcellular organelles (lysosomes, peroxisome, Golgi apparatus, mitochondria), where they transport various molecules across the biological membranes in an ATP-dependent manner [20, 66, 67]. Moreover, expression of the ABCB1, ABCB8, ABCC1, ABCB5 and ABCG2 (Breast cancer resistant protein) have been associated with resistance to chemotherapy in many cancers including melanoma and non-melanoma skin cancers (NMSC) (see Table 1) [1, 14, 61, 68-71]. Nevertheless, PDT has been successful in treating NMSC [72]. In melanoma, although it has produced promising results, resistance has developed as a result of pigment (melanin) interference with light and energy absorption, as well as sequestration of the PS within the double membrane melanocyte- specific vesicles called melanosomes [20, 23].

In PDT resistance specifically, overexpression of ABCG2 has shown to lead to efflux of photosensitizers including hypericin, pheophorbide a, pyropheophorbide a, chlorin e6, 5-aminolevulenic acid (5-ALA) and protoporphyrin [22, 73-75]. The importance and specificity of the ABCG2 transporter to efflux photosensitizers was emphasised in a recent study where it was shown that inhibition of the ABCG2 transporter with non-toxic Ko-134 (analogue of fumitremorgin C) [74] increased the efficacy of PDT in human keratinocytes (HaCat cells). This result was supported by Liu *et al*. (2007) who showed that inhibition of the ABCG2 transporters using imatinib mesylate's, resulted in increased intracellular accumulation of the PS (2-devinyl-pyropheophorbide a, protoporhyrin IX and benzoporphyrin derivative monoacid ring A) within basal cell carcinoma cells (BCC) (ABCG2 positive) and not within squamous cell carcinomas (ABCG2 negative). This increase of PS within the BCC correlates with increased phototoxicity and selectivity, resulting in tumour destruction [76].

An aspect that cannot be ignored that contributes significantly to the therapeutic efficacy of a treatment modality such as PDT is the intracellular localisation of the photosensitisers. The fact that ABC transporters are found localised on these subcellular membranes mean that their roles in therapeutic resistance with respect to organelle localisation need to be investigated. One example is their localisation in melanocyte-specific organelles called melanosomes. These organelles house the process of melanogenesis and subsequent accumulation of the pigment melanin. The melanosome is surrounded by a double membrane due to the toxic intermediates produced during the process of melanin formation. We, and others have shown that pigmented melanoma was more resistant to hypericin activated photodynamic therapy (HYP-PDT) when compared to non-pigmented melanoma [23] and suggested that this was partly due to the pigment melanin, as temporary removal of the melanin using Kojic acid sensitized the cells to the HYP-PDT treatment. This correlated with increased reactive oxygen species (ROS), thus unveiling the scavenging property of the pigment melanin and mature melanosomes [23]. This differential sensitivity to PDT was further corroborated by both Sparsa *et al*. (2013) and Jendzelovský *et al*. (2009) who showed that the combination of the pigment melanin [77] and up-regulation of ABCG2, [78] cause resistance in colon cancer to HYP-PDT. Upon pre-treatment with proadifen (affecting ABCG2 function), this resistance was reversed due to increased intracellular level of HYP and ROS, which subsequently resulted in mitochondrial membrane damage and concomitant cell death [78].

A further example of resistance to HYP-PDT by pigmented and unpigmented melanoma (Figure 1) stems from our work which showed localisation of HYP within the lysosomes, endoplasmic reticulum (ER) and melanosomes in pigmented melanoma [79]. This was well supported by earlier work from Chen and colleagues [62] who showed that the trapping and export of the cytotoxic drug cisplatin by the melanosomes could result from over-expressing ABC transporters, which along

Table 1. Overview of the expression profile of ABC transporters in normal and cancer cells.

Transporter	Cell line	Location	Expression	Drug	Reference
ABCB5	HEM; G3361; CDDP	7p24	Plasma membrane	None	[83]
ABCB1 (MDR1)	CS, H14, JR8	7p21	Cytoplasm	Doxorubicin	[84]
ABCC1 (MRP1)	CS, H14, JR8	16p13.1	Cytoplasm	Doxorubicin	[84]
ABCB8 (mABC1)	WM1552C; 451lu; WM793B	7q36	Inner mitochondrial membrane	Doxorubicin	[68]
ABCB5	WM2664 WM115; G361; A375; SKMEL28	7p24	Cell surface	Temolozide, DTIC; Doxorubicin	[61]
ABCB5	HEM; G3361; CD133	7p24	Cell surface and tissue	Doxorubicin	[83]
ABCG2 (MRP2) or (BCRP)	A375; G2; MRP1; HEK293; KB.V1	4q22	Cell surface	Vemurafenib	[85]
ABCG2	HaCaT	4q22	Not specified	Porphyrin	[74]
ABCG2	NC.H1650; 1650 MX50; MCF7.TX200; MCF7.MX100; MCF7.VP	4q22	Cell membrane	Pyropheophrbid Chlorin 6; hematoporpyrin IX	[22]
ABCG2	RIF-1; Colo 26; BCC-1/KMC; HEK293; MCF7/MR; MCF7FLV1000; MF-10A	4q22	Cell surface	Protoporphyrin X; (BPD-MA); imidazoacridino-ne; topotecan	[56, 81]
ABCC1 (MRP1)	HT-29; HL60	16p13.1	Cell surface	Hypericin	[78]
ABCG2 (BCRP)	HT-29; HL60	4q22	Cell surface	Hypericin	[78]

with melanin content determined cell death via apoptosis or autophagy [77, 80]. A further study contributing to the hypothesis of ABC transporters expressed on subcellular organelle membranes being important to cancer treatment resistance was published by Goler-Baron *et al*. (2012) who demonstrated that resistance in breast cancer was developed when ABCG2-rich extracellular vesicles and lysosomes trapped the photosensitive drugs imidazoacridinone and topotecan (In breast cancer), thus preventing them from reaching their therapeutic targets [70, 81]. Reversal of this resistance was achieved upon illumination of these ABCG2-rich extracellular vesicles and the lysosomes containing the drugs, resulting in severe membranous damage due to the production of reactive oxygen species [81]. This is of particular relevance, as resistance to chemotherapeutic treatment in melanoma resulted from ABCB5 and ABCB1 over-expression, which was found to correlate with cancer progression and aggressiveness. Moreover, it has lately been reported that resistant populations over-expressing

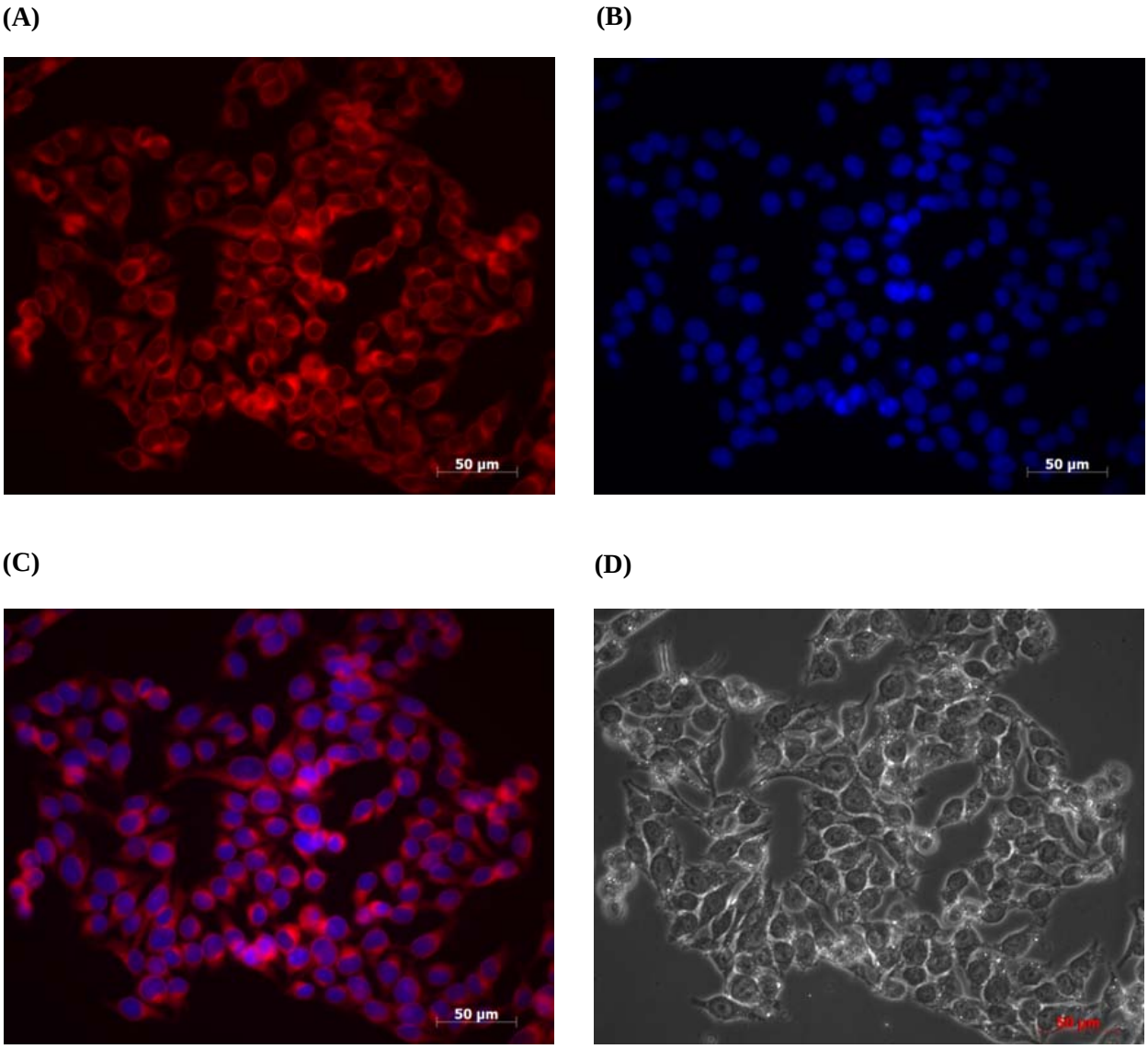

Figure 1. Morphology of A375 unpigmented melanoma cells, 24 hours post-hypericin induced photodynamic therapy (HYP-PDT): A375 cells were exposed to 3 µM HYP-PDT and stained with Hoechst nuclear dye 24 hours post-treatment. Images were captured with an inverted fluorescent microscope showing intracellular HYP (red) **(A)**; nuclear material (blue) **(B)**; HYP and nucleus **(C)**; and the corresponding phase contrast image **(D)**. Magnification: 400x; Scale bar = 50 µM.

ABCB1/5 transporters have stem cell-like properties, which include self-renewal capacity, spheres forming ability as well as yielding differentiated progeny (having the ability to recapitulate the phenotype of the parental tumour, contributing to the heterogeneity of the tumour which can be associated with the relapse observed clinically) [69, 82].

Undoubtedly approaches to overcome the ABC transporters in skin cancer resistance to PDT are needed. One of these approaches could be combination therapy i.e. a chemotherapeutic drug in combination with PDT. Ahn *et al*. (2013) showed that combining Cisplatin with 5-ALA photodynamic therapy (both effluxed by ABCG2) led to greater tumour destruction both *in vitro* and

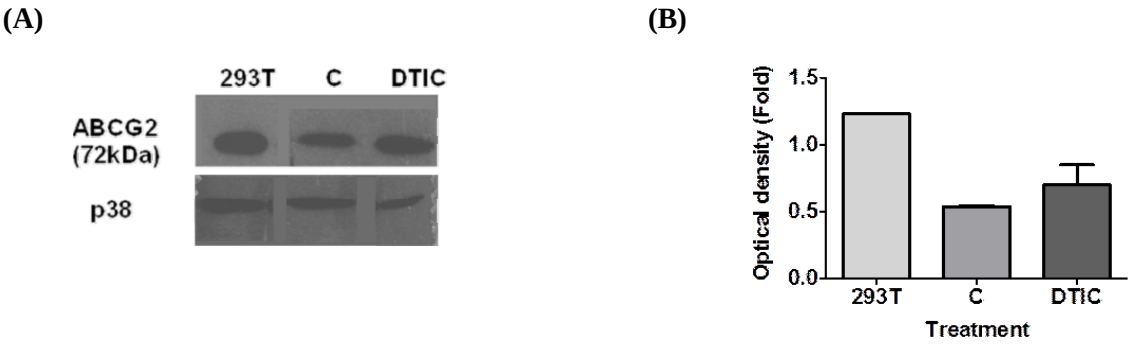

Figure 2. ABCG2 protein expression in unpigmented metastatic melanoma cells (A375), 24 hours post-Dacarbazine (DTIC) treatment: Western blot of ABCG2 protein expression in 293T cells (positive control, human embryonic kidney cells), C (untreated control) and dacarbazine (DTIC) treatment (24 hours) **(A)**. Densitometric analysis showing the optical density (fold) of ABCG2 transporters 24 hours post-DTIC treatment. Data was normalized to the control by obtaining the ratio of ABCG2 expression (band optical density) and its loading control, p38 **(B)**. No statistical significance was obtained using the student t-test ($P < 0.05$) ($n = 3$).

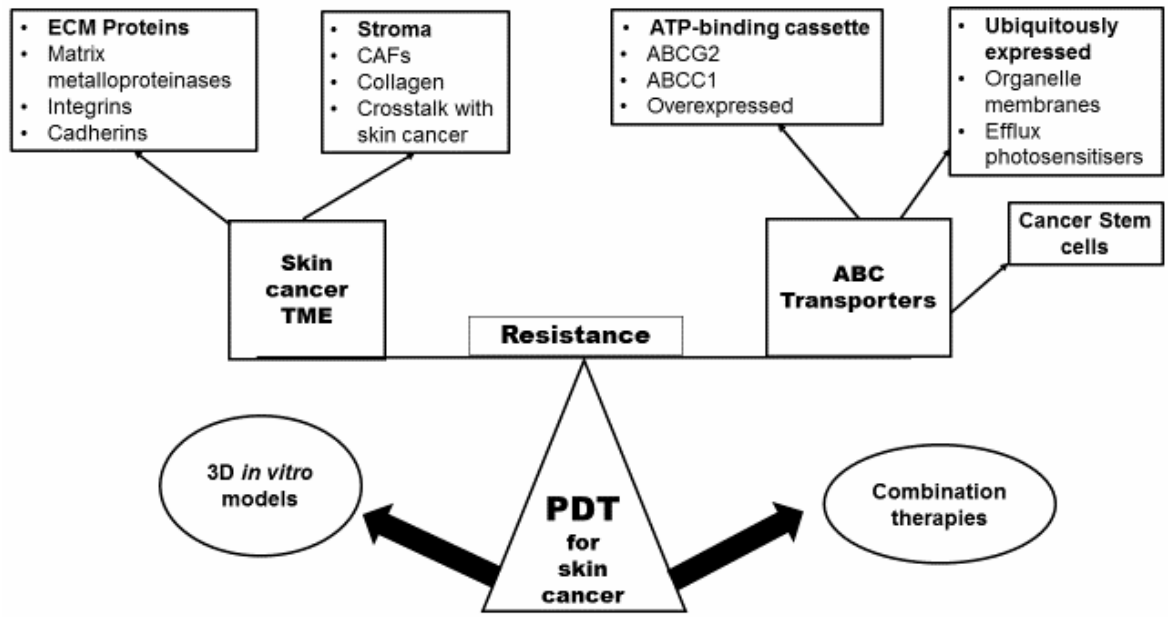

Figure 3. Summary diagram highlighting the considerations to be taken into account for improving the efficacy of PDT.

in vivo, when compared to individual treatments in squamous cell carcinoma [63]. This had the advantage of not only having greater tumour destruction, but also lowered the required concentration of the chemotherapeutic drug [63]. Other treatments involved pre-treatment of melanoma or NMSC tumours with proadifen or verapamil (ABCG2 transporter inhibitor), and then performing HYP-PDT or pre-treating the cells with bafilomycin A1 to prevent lysosomal localisation of the drugs followed by application of PDT treatment. In our own work, we have combined the FDA-approved drug dacarbazine (DTIC) and HYP-PDT in an attempt to increase the efficacy of treatment (Figure 2).

CONCLUSION

Overall, combating skin cancer remains a challenge. Despite recent advances in both the fields of

molecular and clinical medicine, the heterogeneity of this dreaded disease milieu defies most therapies. It is with this in mind that more efficacious, less invasive, pertinent therapies need to be considered. Photodynamic therapy has been touted as one such therapy where the use of a light activated photosensitizer can be a useful tool with minimal invasion but maximal outcome in terms of cellular destruction. Despite limitations such as the need for the presence of oxygen and the timing of exposure of photosensitisers for maximal uptake into a tumor, a number of aspects can be considered to increase its efficacy (Figure 3). This review highlights 2 such aspects. Firstly, that the tumor microenvironment (TME) and its central role in increasing therapeutic resistance must be considered a target for PDT and thus the more we understand about its constitution, the more efficacious we can design our therapies. Lastly, further investigation into the role of ATP-transporters as major players in therapeutic resistance is pertinent if we plan to make significant inroads into the treatment and eradication of skin cancers.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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