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Review

Approaches for targeting mitochondria in cancer therapy☆

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ABSTRACT

The recognition of the role that mitochondria play in human health and disease is evidenced by the emergence in recent decades of a whole new field of "Mitochondrial Medicine". Molecules located on or inside mitochondria are considered prime pharmacological targets and a wide range of efforts are underway to exploit these targets to develop targeted therapies for various diseases including cancer. However the concept of targeting, while seemingly simple in theory, has multiple subtly different practical approaches. The focus of this article is to highlight these differences in the context of a discussion on the current status of various mitochondria-targeted approaches to cancer therapy. This article is part of a Special Issue entitled: Bioenergetics of Cancer.

1. The concept of targeting: appropriate use of "target" and "targeting"

Currently the terms target and targeting are used so commonly that to question their meaning might seem strange. However, for the purpose of this review we wish to first offer our thoughts on the concept of targeting and appropriate use and context of the terms targeting and target. To begin with, it must be appreciated that drug therapy at the most fundamental level is the interaction of two molecules. An exogenous molecule administered to a patient and the molecule in the patient that the administered molecule interacts with to initiate a physiological response. In an ideal scenario, the administered molecule interacts with only one physiological molecule and produces a physiological response that improves a patient's condition. In this context it is clear that the term target may be applied to the physiological molecule and the administered molecule is a drug. The concept of targeting on the other hand has multiple definitions and can often be the source of confusion if not communicated clearly.

From a drug discovery perspective, targeting is very often described in terms of the drug molecule's ability to interact only with the target. This concept is best described by the use of the term selectivity and is very different from the concept of targeting from a military perspective where the term arguably first originated. Consider a bullet fired from a gun as an example. The object the bullet is intended to hit is the target, and the act of aiming the gun so the bullet hits the target is what constitutes targeting. The action that the bullet produces is destruction of the target. This action is indiscriminate in that if the bullet hits an object other than the target, that object will be destroyed as well. Using the gunshot analogy to illustrate the drug discovery perspective on targeting would involve firing bullets that only destroy the target but leave the non-targets unharmed. Most approaches to disease therapy have followed such an argument: finding such selective molecules has been relatively easy when there were significant differences between the disease causing process and normal human biochemical pathways. Not surprisingly, infectious diseases are relatively easier to treat than inherent disorders. The selectivity is however dose dependent and most drugs that are considered to be selectively toxic to invading pathogens are in fact toxic to human cells as well, but at higher doses.

The current challenges in drug therapy lie in the treatment of diseases associated with malfunctions of normal human biochemical pathways in certain tissues. More often than not, even dose dependent selectivity is hard to achieve. Therefore the concept of targeting is becoming more and more associated with selective delivery. The term 'targeting' should ideally imply that the molecule is in some way able to selectively accumulate at an intended site of action and that the selective accumulation is associated with its selective action. This distinction is particularly important in developing targeted therapy for a disease like cancer. Unless unique molecular targets found exclusively (or at sufficiently higher levels) in the diseased state and not in normal state are discovered, selective accumulation at the disease site is crucial to the improvement of therapy. In summary, it can be said that there appear to be two distinct approaches to targeting in the context of drug therapy. The

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first involves selective action on the target while the second involves selective accumulation at the target. Most if not all examples of targeting seem to end up being the combination of some degree of selective action on the target and some degree of selective accumulation at the site of the target. Improving the degree of selective accumulation has the added advantage, even for molecules with high target selective action, of reducing the required dose and hence should be a major focus of all targeting approaches.

In the context of drug molecules the properties of selective accumulation are associated with the concept of bioavailability and biodistribution that are related to the physico-chemical properties of the molecule. To overcome the limitations that a compound's physico-chemical properties can impose on its potential pharmaceutical application, the process of large-scale screening of chemical libraries has been extended beyond identifying desired bioactivity. Screening approaches routinely incorporate selection for physico-chemical properties that are known to confer high bioavailability as well. Unfortunately, this approach often leads to many potent molecules being excluded from further development. These molecules often have a potent pharmacological action at a desired molecular target but aren't able to find their way exclusively to that target. It is almost certain that there is a growing list of such molecules that are in essence potential drugs if only a delivery strategy can be devised to get them to their molecular target in the human body.

Fig. 1 is a schematic representation of the levels of targeting that might be necessary in the treatment of cancer by a targeting approach. After systemic administration, the drug has to reach the tumor mass that is composed of tumor cells and the supporting stroma. The stroma, in turn, is made up of connective tissue, blood vessels, and other non-malignant cells. Therefore, drug accumulation in a solid tumor is only the first step in selective action against cancer. The drug still has to reach the tumor cell, and once inside the cell, it has to reach its final sub-cellular target. The sub-cellular target might be a cytosolic molecule or more often than not, might be a molecule that is on or inside a membrane bound organelle. In the latter case the drug must also be able to enter the organelle and then find its molecular target. Currently, drug targeting is well accepted till the cellular level as evidenced by the large number of approaches being explored to achieve cell-specific accumulation of drugs. However, targeting at a sub-cellular level has until recently not been as widely pursued perhaps due to technological limitations or the argument that once a drug gets inside a cell it will eventually find its way to the sub-cellular target. In the context of cancer therapy mitochondria are widely recognized to be the location of several potential drug targets.

2. Mitochondrial targets for cancer therapy

The mitochondrion is an important organelle that mediates several critical processes in a eukaryotic cell. Of prime importance in the physiology of cancer is the role of mitochondria in energy metabolism and regulation of cell cycle. There is strong evidence to support the rationale for the development of anticancer strategies based on mitochondrial targets. Mitochondria are known to play a key role in the complex apoptotic mechanism and trigger cell death via several mechanisms that include disrupting electron transport and energy metabolism, releasing or activating proteins that mediate apoptosis and altering cellular redox potential. [1–3]. A critical event leading to programmed cell death is the mitochondrial membrane permeabilization, which is under the control of the permeability transition pore complex (mPTPC), a multiprotein complex formed at the contact site between the mitochondrial inner and outer membranes. Apoptosis plays a central role in tissue homeostasis and it is generally recognized that inhibition of apoptosis contributes to the transformation process of normal cells into cancer cells [4]. The dysfunction of most apoptosis regulating pathways has been found to be linked to various types of cancer [5,6]. The key role of mitochondrial dysfunction and altered apoptotic regulatory mechanisms has been appreciated for more than a decade [7–10]. Closely allied with the dysregulation of mitochondrial involvement in the apoptotic process is the altered role of mitochondria in the energy metabolism of malignant cells [11]. Cancer cells are known to favor the glycolytic process as a source of ATP, even under aerobic conditions. Such adaptations are believed to contribute to invasive and adaptive advantages and are often the result of changes in mitochondrial function including mutations in mitochondrial DNA (mtDNA) [12]. Therefore this organelle is increasingly described as a “prime target” for pharmacological intervention [13] and there is a growing interest in the study of the molecular interactions of xenobiotics with cellular components located on or inside the mitochondrion. Research by several groups has identified various mitochondria associated molecular targets for bioactive molecules [14–18]. These targets include mtDNA, the mitochondrial respiratory chain, the mitochondrial permeability transition pore complex (mPTPC), potassium channels on the mitochondria and the various mitochondria associated anti and pro-apoptotic factors to name a few [13,14,19]. An exhaustive discussion of the various mechanistic pathways and all potential target molecules in mitochondria of cancer cells has been the subject of several excellent reviews published over the past few years [11,20–22] and as such will not be the major focus of this article. More relevant to the intent of this article is the discussion of current examples of so-called mitochondrial targeting and how they fit into the concept of targeting as discussed in the previous section. With this in mind, two major approaches can be envisioned for targeting mitochondria in cancer cells. The first involves the use of molecules that act exclusively on molecular targets in mitochondria of cancer cells without having any marked predisposition for preferentially accumulating in mitochondria. The second approach involves delivering molecules capable of affecting mitochondrial function exclusively to the mitochondria of cancer cells. Of course as discussed earlier the two approaches need not be exclusive with the best

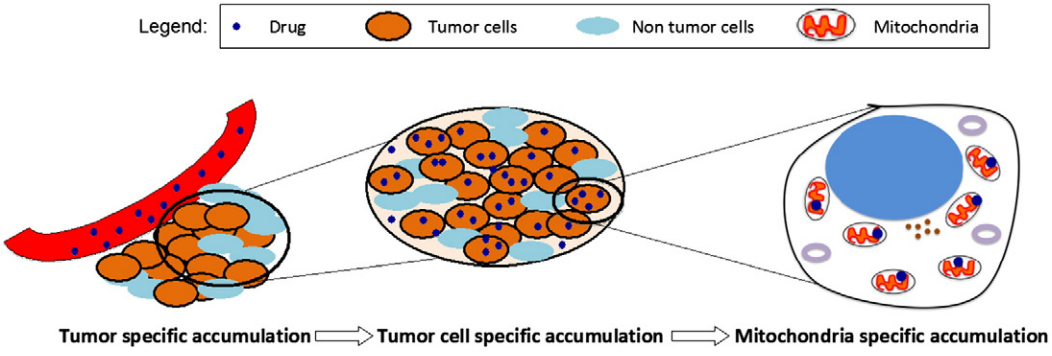

Fig. 1. A schematic representation of the levels of selective accumulation required in a mitochondria-specific targeting strategy.

scenario being the use of a drug with highly selective action on the mitochondria and a delivery strategy aimed at achieving its mitochondria-specific accumulation in cancer cells.

3. The selective action approach to targeting mitochondria of cancer cells

There are numerous molecules currently in use or being tested in clinical trials that act on mitochondria [11]. Several clinically approved anticancer drugs such as paclitaxel [23–25], VP-16 (etoposide) [26] and vinorelbine [25] as well as an increasing number of experimental anticancer drugs such as, ceramide [27], MKT077 [28] and CD437 [29–31], lonidamine [32,33], betulinic acid [34–36] have been found to act directly on mitochondria to trigger apoptosis [37]. CD437 is able to induce apoptosis in a variety of human carcinoma cells *in vitro* and *in vivo* [38]. In intact cells, CD437-dependent caspase activation is preceded by the release of cytochrome C from mitochondria [31]. Moreover, when added to isolated mitochondria, CD437 causes membrane permeabilization. This effect is prevented by inhibitors of the mitochondrial permeability transition pore complex (mPTPC), such as cyclosporine A. Therefore, CD437 represents a low molecular weight compound, which exerts its cytotoxic effect via the mPTPC, i.e., by acting directly at the surface or inside of mitochondria. Similarly, arsenic trioxide, which is used in the treatment of acute promyelocytic leukemia, has multiple actions on mitochondria. It is known to cause the induction of mPTPC formation via its action on the voltage-dependent anion channel VDAC [39]. Arsenic trioxide is also known to act on the respiratory chain and inhibit respiratory chain activity [40].

Apoptotic factors that play a major role in the modulation of apoptosis include Bcl-2 and Bcl-X_l. Compounds that act by binding to these proteins have been identified and studied for their efficacy, a few examples include a chromene derivative [41], and gossypol which was recently shown to act on proteins of the Bcl-2 family [42]. In fact there are so many varied and structurally different compounds that it has been suggested that they be collectively called mitocans to reflect their mitochondrially mediated anticancer effects [43–45].

Unfortunately, despite the seemingly large list of compounds that can interfere with mitochondrial function [11], it is still unclear as to what extent these molecules can selectively accumulate at the tumor. Therefore most, if not all, of the molecules described so far can potentially benefit from selective delivery to the tumor. For example the rhodacyanin compound MKT-077 was investigated in clinical trials on the basis of its ability to selectively accumulate in mitochondria and inhibit ATP synthesis [28,46]. Despite exhibiting a prolonged retention in mitochondria of cancer cells compared to non-cancer cells, the drug was found to cause significant nephrotoxicity with a mechanism related to mitochondrial dysfunction [28]. The drug was therefore rejected from phase II trials. It is very likely that the toxicity resulted from the distribution of significant portions of the administered dose to non-tumor sites. If so, a tumor-specific delivery strategy could improve the therapeutic prospects for this drug.

In the case of molecules that do not exhibit the marked mitochondria-specific accumulation of MKT-077, tumor-specific delivery must further be combined with a strategy to achieve mitochondria-specific accumulation once inside the cell. Although not directly related to an anticancer strategy Cyclosporin A (CsA) provides a good example of a drug that requires a mitochondria-specific delivery strategy. CsA has been shown to bind with nanomolar affinity to mitochondrial cyclophilin D, which potentially makes it an interesting antiischemic drug candidate [47]. However, CsA also targets various other cyclophilins inside the cell, which are each likely to bind some portion of the administered drug. Therefore, the mitochondrial concentration of CsA is difficult to predict and an effective CsA treatment may require high, even toxic concentrations to elicit an effect at the mitochondrial target [48]. Consequently, a mitochondria-specific delivery approach would potentially improve the therapeutic benefit of CsA [49]. Similar scenarios exist among the anticancer drugs. Paclitaxel, for example is generally known as an anti-microtubule agent but has also recently been demonstrated to trigger apoptosis by directly acting on mitochondria [23–25]. Clinically relevant concentrations of paclitaxel can trigger apoptosis by inducing cytochrome C release in a mPTP-dependent manner. This mechanism of action is also associated with other pro-apoptotic agents known to directly act on mitochondria [35]. A twenty four hour delay between the treatment with paclitaxel or with other PTP inducers and the release of cytochrome C in cell-free systems compared to intact cells has been attributed to the existence of several drug targets inside the cell and only a fraction of the total intracellular drug being available for action on mitochondria [23]. Anti-tubulin agents such as vinorelbine or nocodazole have been shown to trigger the release of cytochrome C via the direct interaction with mitochondria [50], which subsequently resulted in apoptotic cell death. Etoposide which is a mitochondrial topoisomerase inhibitor, is known to act at a higher concentration by inducing the release of apoptotic factors while at a lower concentration it acts by causing nuclear DNA damage [51].

Therefore, in the more than likely scenario that potential drug molecules do not exhibit adequate tumor and mitochondria-specific accumulation, a concerted effort to develop tumor-targeted mitochondria-specific approaches might be significant aid towards exploiting mitochondrial targets for cancer therapy.

4. The selective accumulation approach to targeting mitochondria of cancer cells

As illustrated in Fig. 1 the selective accumulation approach to targeting tumor mitochondria requires two levels of specific accumulation; drug accumulation in the tumor and then drug accumulation in the mitochondria of cancer cells. Generally speaking, drug disposition may be modulated by subtle modification of the chemical structure to change its physico-chemical properties that are known to determine its accumulation in various compartments. Of course such modification must be done without adversely affecting action on the molecular target. The second approach involves conjugating ligands that are larger than simple organic functional groups to change the biodistribution of the active molecule. Again this approach works as long as the conjugation does not adversely affect the desired pharmacological activity of the molecule. Such approaches have been used very effectively to alter drug distribution in the body and achieve higher accumulation in target tissues using ligands that are known to have an affinity for the target tissue. There are ligands that have been shown to mediate tumor-specific accumulation of drugs, and there are ligands that are known mitochondriotropics. However it is still unclear whether there exists a ligand that possesses both properties to a degree that will allow high levels of the desired accumulation. It may therefore be safe to say that, for now, a dual strategy is the most feasible approach. Such a dual strategy would require the use of one targeted delivery approach to achieve high tumor accumulation followed by a second approach to ensure that the drug then accumulates in the mitochondria where it will exert its action. While there is much investigation into tissue-specific delivery aimed at increasing tumor levels of anticancer drugs, research aimed at sub-cellular delivery is only just gaining more attention [52,53]. Nonetheless there are some interesting approaches to mitochondrial delivery that suggest the promise of improved therapy for cancer.

4.1. Molecular modification approaches for selective accumulation in mitochondria

The interior of a cell is very different from a dilute aqueous solution, in which small drug molecules can freely diffuse and

randomly interact with other dissolved molecules. The presence of the cytoskeletal network, discrete organelles and a multitude of macromolecules [54,55] and other dissolved biomolecules produce a highly-constrained environment much different from that in which classical Fickian diffusion is likely to be the dominant mode of non-specific transport [56,57]. The fluid-phase viscosity of the cytoplasm, and the binding to intracellular components are also believed to influence the diffusion of solutes inside a cell [58,59] and efforts towards understanding such parameters are already underway [60]. Properties of the medium aside, it is generally accepted that the physico-chemical properties of the drug also play a major role in determining the sub-cellular fate of the drug molecule. Therefore, the ability to predict the influence of various properties of the drug molecule on the likely site of accumulation within the cell is a powerful tool to either select molecules with the desired mitochondrial accumulation or identify molecules that would benefit from mitochondria-specific delivery strategies. To this end, a Quantitative Structure Activity Relationship (QSAR) model for predicting cellular uptake and intracellular distribution of low molecular weight compounds has been proposed [61]. This QSAR approach was recently applied to identify potential common chemical features of molecules that are known to selectively accumulate at or inside mammalian mitochondria within living cells [62]. The QSAR approach has additionally proven useful for the modeling of cationic transfection lipids [63] and could therefore be applicable to predicting the sub-cellular disposition of a potential therapeutic molecule and even to assist in the selection of structural modification of therapeutic molecules to impart the desired mitochondrial affinity. A survey of the literature reveals that the most commonly used mitochondrotropic molecule is the triphenylphosphonium (TPP) cation. Conjugation of a TPP cation has been used to deliver various bioactive cargos to mitochondria including antioxidants like coenzyme Q, ubiquinone and various nitroxides [64]. The TPP cation was also used to deliver peptide nucleic acids [65] and Cyclosporin A to mitochondria indicating the broad applicability of such an approach to mitochondria-specific delivery. However, more recently and of more direct relevance to the discussion on cancer therapy is the use of the TPP cation to modify porphyrins for potential mitochondria-targeted photodynamic therapy. Meso-tetraphenylporphyrin derivatives bearing either the triphenylphosphonium ion or the triethylammonium ion were shown to be mitochondria-targeted and to exhibit light dependent toxicity in MCF-7 human breast cancer cell line, suggesting their application potential in photodynamic therapy. Additionally the triphenyl phosphonium cation and various other mitochondriotropic residues were used to synthesize several examples of a class of molecules described as geldanamycin mitochondrial matrix inhibitors (Gamitrinibs). Gamitrinibs are designed to selectively target and inhibit Hsp90 activity in mitochondria of human carcinoma cells by acting as ATPase antagonists. Most interesting is the claim that gamitrinibs are non-toxic to non-cancer cells and have no effect on Hsp90 function in non-mitochondrial sub-cellular compartments. The drug TEMPOL also provides a very interesting example of the application of a conjugation approach to improve mitochondrial targeting. TEMPOL is a piperidine nitroxide that acts as a prodrug and is reduced to a hydroxylamine in mitochondria by ubiquinol. The metabolite induces apoptosis in cancer cells by action on complex I [66]. Conjugation of a TPP residue to TEMPOL improved its mitochondria-specific accumulation and its reaction with ubiquinol in mitochondrial membranes [67].

In addition to the so-called delocalized cations [68] like TPP, rhodamine and various other mitochondria-specific stains, there exists another distinct class of mitochondria-specific delivery ligands. The various mitochondrial matrix proteins that are synthesized in the cytosol and imported into the matrix by the mitochondrial import machinery rely on short leader sequences to mediate this specific import process. Just like the TPP cation, the leader sequences of various matrix proteins have also been used to mediate the mitochondria-specific accumulation of a variety of bioactive molecules [69]. Based on the rationale that short peptides can exhibit mitochondria-specific localization, the pro-apoptotic and potential anticancer peptide $(KLAKLAK)_2$ was engineered to improve mitochondrial localization [70]. Further, a recent report that conjugation to bombesin enhanced the cytotoxicity of three mitochondria-disrupting peptides through improvement of their binding affinity makes a case for the conjugation approach in improving the tumor association of agents that already possess mitochondria-specific action [71]. This raises an interesting question of whether or not it is possible to have a ligand that can mediate both tumor-specific delivery and mitochondria-specific delivery inside the tumor cells. This may still be asking too much from a single molecule. There exist three alternatives. The first is the use of a dual conjugation strategy with two ligands conjugated to the same active molecule. Second is the use of a tumor-specific nanocarrier to deliver an active molecule that has been conjugated to a mitochondria-specific ligand. The final alternative is the use of a tumor-specific and mitochondria-specific nanocarrier. The development of nanocarrier-based approaches thus becomes an integral part of mitochondria-targeted approaches to cancer therapy.

4.2. Nanocarrier based approaches for selective accumulation in mitochondria

Pharmaceutical nanocarriers like liposomes and micelles, solid nanoparticles offer what might be viewed as a non-chemical approach to modify the disposition of drug molecules. All chemistry can be performed on the components of the nanocarrier system that can then be loaded with the drug to afford targeted delivery [72–75]. Most pharmaceutical nanocarriers can be modified for targeting to specific tissues and even specific cell types. Long-circulating liposomes and nanoparticles are able to passively target areas of leaky vasculature by virtue of the enhanced permeability and retention (EPR) effect and can additionally be modified with antibodies or other targeting ligands to afford cell-specific recognition [76–80]. Nanocarriers that cannot only effect the tumor-specific accumulation of a drug but also mediate mitochondria-specific accumulation within a tumor cell might be the ultimate tool in mitochondria-targeted anticancer approaches if they can be developed for clinical therapy. The first steps in this direction have already been taken in recent years. Current nanocarrier technology is reaching the point where the need for sub-cellular delivery may indeed be met using nanocarriers specifically designed for such purposes.

In an approach towards the delivery of the mPTPC inducing drug mastoparan into cells [81] liposomes were modified with both transferring and a fusogenic peptide Chol-GALA. The transferrin modification enhances liposomal uptake into cells via endocytosis after which the peptide facilitates release from the endosomes into the cytosol. Thus, by just increasing the intracellular content of the drug, the delivery approach achieved a higher concentration of the drug that was potentially available to interact with the sub-cellular target. Micelles have also been proposed for the delivery of hydrophobic drugs to various sub-cellular organelles including mitochondria [82]. The fluorescently labeled micelles used in the study were found to be distributed through several cytoplasmic organelles including a majority of them being associated with the mitochondria. The uptake of these micelles was not restricted to a single cell type. Also, the extent of cell internalized cargo incorporated in micelles was greater than the free cargo by itself. There are now several examples of nanocarriers designed specifically to accumulate in mitochondria. Arguably the earliest of these are what are known as DQAsomes. Prepared from the mitochondriotropic molecule dequalinium chloride, these vesicular nanocarriers were developed for mitochondria-specific DNA delivery but were also shown to be capable of changing the sub-cellular distribution of paclitaxel to

increase the accumulation of the drug in mitochondria. The mitochondria-specific delivery led to improved apoptotic activity at paclitaxel concentrations, at which the free drug does not have a significant cytotoxic effect [83]. Paclitaxel loaded DQAsomes have also been tested for their ability to inhibit the growth of human colon cancer tumors in nude mice [84] and the data strongly suggest that encapsulation of paclitaxel in DQAsomes leads to improved efficacy. The antitumor efficiency of DQAsomal encapsulated paclitaxel was also further enhanced by modifying the DQAsomal surface with folic acid (FA) [85]. The folate receptor is a folate high-affinity membrane binding protein, which is overexpressed in a large variety of human tumors [86–88]. FA conjugates are internalized in a tumor cell-specific manner by receptor-mediated endocytosis resulting in an increased toxicity of the corresponding drug [89–91]. Cell cytotoxicity studies using folate receptor expressing HeLa cells suggested that folic acid conjugated DQAsomes possess better antitumor activity as compared to plain paclitaxel loaded DQAsomes, folic acid conjugated paclitaxel loaded liposomes and the free drug. Based on the data it was concluded that folic acid conjugated DQAsomes delivered the drug not only to the cytosol but also to mitochondria whereas folic acid conjugated liposomes delivered the drug into the cytosol only [85].

Another approach to the design of mitochondria-specific nanocarriers is to modify existing nanocarriers with mitochondriotropic ligands. In this regard TPP again served as the mitochondriotropic ligand in liposomal and polymer based nanocarriers. Liposomes have been well characterized as delivery systems and are a popular choice due to their biocompatibility, ease of surface modification, capacity to encapsulate hydrophilic or hydrophobic drugs [92]. The first indication that liposomes could be rendered mitochondriotropic by surface modification with a mitochondriotropic residue came from a report that so-called proteoliposomes prepared by incorporating a crude mitochondrial membrane fraction into liposomes colocalized with endogenous mitochondria in pre-implantation embryos [93]. Further investigation of the concept of using ligands to alter the sub-cellular distribution of liposomes was based on the synthesis of stearyltriphenylphosphonium (STPP) [94]. The stearyl residue of STPP serves as a lipid anchor to modify the surface of liposomes with the TPP residue and resulted in a liposomal preparation with a marked predisposition for mitochondria [94]. STPP-liposomes were shown to effectively direct the accumulation of rhodamine labeled phosphatidylethanolamine (Rh-PE) to mitochondria in live cells [95]. Based on flow cytometry STPP-liposomes exhibited the same level of cell association as liposomes with the same cationic charge. However, the subsequent sub-cellular localization analyzed by confocal microscopy was markedly different indicating that the mitochondriotropic ligand and not the surface charge is what determines mitochondria-specific association of the nanocarrier [95]. It was also found that the TPP ligand did not change the *in vivo* distribution and tumor accumulation of long-circulating PEGylated liposomes. STPP-liposomes did however improve the both the *in vitro* and the *in vivo* efficacy of ceramide. Taken together these data suggest that the tendency of long-circulating liposomes to passively accumulate (via the EPR effect) in solid tumors can be combined with organelle-specific tropism conferred by modification with an appropriate ligand to potentiate the effect of an encapsulated antitumor agent.

An alternative approach to developing mitochondria-specific liposomes has focused on the concept that liposomes with a tendency to selectively fuse with mitochondrial membranes are more likely to associate with mitochondria upon cell entry. Referred to as MITO-Porter, these liposomes are surface modified with octaarginine residues to facilitate their entry into cells as intact vesicles (via macropinocytosis) [96]. The lipid composition was selected on the basis of high levels of fusion with the mitochondrial membrane and the release of its cargo to the intra-mitochondrial compartment in living cells [96]. Based on confocal microscopy data, MITO-porter liposomes have been used to deliver green fluorescent protein [96] as well as propidium iodide [97] to mitochondria suggesting that they can be used to deliver macromolecules as well as small molecules to mitochondria.

The development of mitochondria-specific nanocarriers has not been limited to lipid based carriers but also includes the use of mitochondriotropic residues to create polymeric systems capable of mitochondria-specific intracellular delivery of bioactive molecules [98–100]. TPP modification has been employed to create a mitochondriotropic *N*-(2-hydroxylpropyl)methacrylamide (HPMA) copolymer-based nanoparticle [99,100]. Interestingly the first study indicated that while the polymers characterized did exhibit association with isolated mitochondria, experiments with ovarian carcinoma cells revealed predominantly lysosomal association of the polymer [99]. However, in the more recent study [100] microinjection and incubation experiments performed using fluorescently labeled constructs suggested mitochondrial targeting ability based on microscopic analysis. Subsequently, HPMA copolymer-drug conjugates were synthesized using a photosensitizer mesochlorin e 6 (Mce 6). Mitochondrial targeting of HPMA copolymer-bound Mce 6 enhanced cytotoxicity as compared to non-targeted HPMA copolymer-Mce 6 conjugates [100]. The authors indicate that "minor modifications may be required to adapt the current design and allow for tumor site-specific mitochondrial targeting of other therapeutic agents" [100]. Therefore these systems could theoretically be applied to the mitochondria-specific delivery of a range of pro-apoptotic substances for cancer therapy.

Inorganic nanoparticles have also been shown to be capable of mitochondria-specific delivery. In a very recent study, hypocrellin A, a photodynamic drug, was encapsulated in a water-soluble amorphous silica nanocage (HANC) [101]. These drug-loaded nanocages are reportedly able to specifically accumulate in the mitochondria of cancer cells and improve the photosensitizing effect of hypocrelin A. [101]. It is however unclear what mediates the mitochondria-specific accumulation of the nanocage system. Nevertheless, taken together, the various studies described so far strongly support the hypothesis that nanocarriers can indeed control the sub-cellular accumulation of bioactive molecules and as such represent a useful tool in the development of mitochondria-targeted anticancer strategies.

5. Conclusions and perspectives

The recognition of the role that mitochondria play in human health and disease is evidenced by the emergence in recent decades of a whole new field of "Mitochondrial Medicine" [102,103]. As discussed so far, targeting mitochondrial molecules in the development of cancer therapy relies on the two basic interpretations of targeting via selective action on the target and selective accumulation at the target site where the term target must represent a molecule and not a particular tissue or cell location. Table 1 provides a summary of current examples on the basis of these broad classes. Irrespective of the level of selective action that a molecule possesses, it can be argued that a selective delivery strategy is desirable. In the case of molecules with ideal selective action, selective delivery allows economical dosing while in the case of molecules with significant off target accumulation and effects, selective delivery also offers improved safety. It must be appreciated that the probability of finding a single molecule with ideal selective effect in diseases like cancer is very low given the very subtle differences in cancer cell-specific targets from normal cells. Even more improbable is the existence of molecules with both ideal selective action and selective accumulation. Therefore drug discovery studies must include approaches aimed at conferring selective accumulation on molecules with promising selective action. Direct conjugation to ligands that mediate the desired tumor and sub-cellular accumulation has been used effectively and therefore offer significant promise. Pharmaceutical nanocarriers offer much promise for tumor-specific delivery and have also recently been explored for sub-cellular delivery. We expect that an ideal approach to mitochondrial targeting in cancer therapy might therefore be represented by

Table 1
A summary of current approaches to targeting mitochondria in cancer therapy.

Approach	Description	Advantages	Disadvantages	References
Selective action	Use of drug molecules that are identified to act on mitochondrial targets	▪ Simplicity of the approach ▪ Relative ease of manufacture ▪ No special delivery system required	▪ Uncontrolled distribution to non-tumor sites ▪ Higher dose requirement ▪ Potential toxicity issues	[23,26,28,29,31,33,35,41,42,45,47]
Selective accumulation Molecular modification	Use of molecules produced by conjugating mitochondriotropic molecules to drug molecules that act on mitochondrial targets	▪ Potential improvement in mitochondrial accumulation ▪ Potential lower dose requirement	▪ Tumor-specific accumulation is not necessarily achieved ▪ Potential loss in drug activity after conjugation	[65,66,70,71]
Selective accumulation Nanocarrier incorporation	Use of mitochondriotropic nanocarriers loaded with drug molecules that act on mitochondrial targets	▪ Potential lower dose requirement ▪ Potential tumor-specific accumulation	▪ Relative complexity of approach ▪ Relative difficulty to manufacture	[84,89,90,93,95,96,98,100,101]

the identification of a molecule with a high level of selective action on the mitochondrial target. The molecule may then be conjugated with a mitochondria-specific ligand and then loaded into a tumor cell targeting nanocarrier, thus conferring in one composite system, all the required levels of selective accumulation and selective action to constitute a truly targeted approach.

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