New strategies in melanoma therapy: can nanoparticles overcome chemoresistance?

“...there are a large number of studies evaluating nanoparticulate systems for overcoming drug resistance at cellular and molecular levels.”

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Melanoma is the deadliest form of skin cancer with the fastest growing incidence rate among all cancers. According to the National Cancer Institute, the incidence rate increased more than 60% in the past 20 years. Despite advances in therapy, the mortality rate remained constant in the same period [1]. Melanoma is extremely difficult to treat due to its high heterogeneity and ability to evade the immune system and develop multidrug resistance; rendering successive therapies ineffective [2]. Thus, there is a continuous need to develop new treatment strategies. The first nanotechnology approaches for cancer therapy were developed to improve the bioavailability and distribution profile of poorly soluble chemotherapeutic agents, leading to the first liposomal doxorubicin formulation on the market. In addition to drug encapsulation, nanoparticles are developed to carry their cargo specifically to tumor sites by passive targeting, capitalizing on the higher permeability of the blood vessels in tumors compared with the healthy endothelium (enhanced permeability and retention effect). To increase targeting specificity and efficiency, nanoparticles can be decorated with targeting moieties, increasing their uptake by the tumor cells and minimizing internalization, hence toxicity, by the surrounding healthy cells. Another advantage of nanoparticulate cancer therapy is that the building elements of the nanoparticles can

be tailored to release their cargo only at the tumor site by responding to the specific conditions surrounding the tumors (e.g., pH, presence of certain enzymes, changes in oxygen pressure) [3]. One of the major benefits of encapsulating the drugs into nanoparticles is their potential for overcoming drug resistance [4]. Tumor resistance to therapy occurs due to complex phenomena including physical barriers, such as poor vascularization and increased tumor pressure; biochemical processes, such as upregulation of efflux enzymes and mutations that render targets inefficient; and, especially prominent for melanoma, intracellular sequestration of the drug that prevents its access to the intracellular target [5]. Nanoparticles can overcome these barriers by increasing drug permeation into solid tumors, using endocytotic pathways for cellular internalization and promoting escape of the endosomes. A significant number of studies address melanoma drug resistance, as novel chemotherapeutic compounds become inefficient as monotherapy quickly after initiation, thereby preventing their use for prolonged and repeated therapies.

Overcoming drug resistance at the cellular level

Several different types of nanoparticles are demonstrating an ability to overcome drug resistance in cellular models. Although not directly addressing drug resistance, Pego-

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raro *et al.* showed that doxorubicin, encapsulated in polymersomes exhibited selectivity to melanoma cells, compared with healthy fibroblasts [6]. Doxorubicin conjugated to PEG-lipids enhanced cellular uptake of doxorubicin and promoted cellular retention twofold by preventing efflux protein activity in multidrug-resistant cancer cells [7]. The same drug, co-encapsulated with ceramide in targeted lipid-based nanoparticles caused 90% cell death in a short 4-h-incubation period in both drug-sensitive and -resistant cells – results unattainable by the free drug or by the liposomal doxorubicin alone. This study demonstrated the ability of the targeted liposomal combination to act synergistically and overcome resistance to doxorubicin [8]. A nanosized polymeric complex of paclitaxel showed reversal of drug resistance by mimicking a catalytic reaction, thereby recovering the ability of the paclitaxel to react with its intracellular target at low concentrations [9]. Our group designed and developed cyclodextrin-conjugated lipid nanoparticles, which increased the efficiency of melphalan and were able to recover the cytotoxic activity of the drug in melanoma cells rendered resistant to the free drug [10]. PAMAM dendrimers could increase cellular permeability and increase paclitaxel efficiency in murine melanoma cells [11]. Paclitaxel, a substrate for the efflux pump P-gp, when co-encapsulated with curcumin in liposomal delivery systems showed up to 50% higher toxicity compared with the combination of free drugs [12]. Recently, new drugs, such as temozolomide were also reported as candidates for encapsulation into dendrimers in order to increase cellular uptake and, without conclusive evidence, potentially enhance retention in melanoma cells [13]. The number and variety of nanomaterials and combination of drugs working synergistically in *in vitro* studies provide a good source for formulation developers to repurpose current drugs for drug-resistant melanoma. Through systematic exploration of the physicochemical and biological properties of these nanoparticles, lead nanoparticle systems are identified that can be assessed in animal models.

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In vivo studies

There is a trend to improve current therapeutic agents through encapsulation in novel nanomaterials. A natural progression in many research groups is to select promising nanoparticulate systems from *in vitro* screening and assess their performance in experimental animals.

Although the *in vitro* models are informative with regard to basic processes, such as cellular internalization, intracellular trafficking and targeting certain intracellular organelles or biomolecules, complex questions regarding pharmacokinetic profile, biodistribution and *in situ* tumor accumulation cannot be answered without the use of experimental animal models. For example, Zou *et al.* described a threefold tumor accumulation of doxorubicin in melanoma-bearing animals using polymeric nanoparticles compared with the pegylated liposomal doxorubicin that is available on the market [14]. A simple, PEG-lipid conjugated doxorubicin system conjugated to integrin-targeting peptide showed a 79% reduction of melanoma nodules compared with the free drug administered intravenously [7]. Polymeric nanoparticles loaded with three anticancer agents, docetaxel, everolimus and LY294002, showed a significant reduction of melanocytes dependent on the charge of the nanoparticles in two different metastatic melanoma models, due to the ability of nanoparticles to overcome drug resistance [15]. These studies highlight that many different strategies can be employed to render drugs effective. For example, conjugation or encapsulation of drugs into multifunctional nanoparticles can act in multiple ways to overcome drug resistance, such as enhanced tumor penetration and cellular uptake increasing intracellular drug concentration; facilitated intracellular trafficking and protection of the drug from intracellular degradation and expulsion. Encapsulation of multiple drugs acting synergistically into nanosized systems to be co-delivered to the tumor could overcome drug resistance by attacking different facets of the problem at the same time. The positive outcomes in these *in vivo* studies are an illustration that there is a potential for nanoparticle-based approaches to reach clinical development stages.

Clinical trials

Currently, there are approximately two dozen interventional clinical trials in the USA addressing specifically advanced resistant melanoma [16]. Out of these trials, the majority employ combination therapies to mitigate various facets of the condition, such as NCT00925132 using temozolomide, decitabine and panobinostat, NCT01876641 using vemurafenib, cobimetinib and decitabine and NCT01619774 with dabrafenib–trametinib combination. These studies are mostly Phase I and II studies assessing safety and efficacy in a limited number of patients. Out of the available outcomes, the dabrafenib–trametinib combination showed clinically significant improvement only in 15% of participants with a median progress-free survival of 13 weeks [17]. The temozolomide–decitabine–panobinostat treatment proved to be well tolerated in the Phase I trial, although the main reason for discontinuation was dis-

ease progression [18]. The limited improvement of the combination therapies indicates that there is a need to re-address resistant malignancies from a different angle, such as nanotherapeutics. There is one study using nanoparticulate formulation in patients with resistant tumors: NCT00703170, a Phase I study evaluating the safety and tolerability of pegylated liposomal doxorubicin with or without temsirolimus. Pegylated liposomal doxorubicin is used in a Phase III trial, NCT02580058 in another resistant solid tumor, ovarian cancer. Liposomal vincristine is used in a Phase I pharmacokinetic study in melanoma (NCT00145041) and in a Phase II study in uveal melanoma (NCT00506142). Paclitaxel–albumin nanoparticles are used in several melanoma studies alone or in combination with chemotherapeutic agents and biologicals. It is encouraging that a number of clinical trials involve nanomaterial-conjugated chemotherapeutic agents. Although the primary objectives revolve around improvement of safety and efficacy, the intrinsic ability of some nanoparticulate therapeutic agents to overcome drug resistance can add benefit to these therapies.

Nanotechnology drugs on the market

Out of the approved nanotechnology products on the market including albumin-conjugated and polymeric paclitaxel, liposomal formulations of doxorubicin, daunorubicin, cytarabine and vincristine, none are indicated for melanoma. However, currently conducted clinical trials assessing the safety and efficacy of nanoparticulate systems may lead to approval of some of these therapeutics for drug-resistant melanoma.

Conclusion

As current therapies on the market show limited success in combating melanoma, especially drug-resistant malignancy, there is a need to develop novel strategies for mitigating the disease. Certainly there are hurdles to overcome in the process of development, such as stability and reproducibility issues with the nanoparticulate formulations; poor *in vitro/in vivo* correlation; slow development of appropriate animal models; regulatory barriers; and failure of the drugs in early clinical trials. Yet, there are a large number of studies evaluating nanoparticulate systems for overcoming drug resistance at cellular and molecular levels. Some of these nanoparticles performed well in preclinical studies, demonstrating favorable pharmacokinetic and pharmacodynamic profiles. The success of the liposomal formulations on the market demonstrates that the path is established and, similarly to the explosion of biological products on the market, nanotechnology products may become the treatment of choice for melanoma resistant to chemotherapeutic agents.

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