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Paying “particle” attention to novel melanoma treatment strategies

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Summary

Malignant melanoma remains the deadliest form of skin cancer due to its highly aggressive nature and the lack of effective treatments to combat it. Recent investigations into alternate melanoma treatment strategies have highlighted the exciting potential of nanoparticles in increasing melanoma cell delivery and efficacy of small interfering RNAs (siRNAs) or pharmacological inhibitors. In this issue, Chen *et al.* formulate a new liposomal nanoparticle for c-Myc siRNA delivery and find it highly effective in reducing c-Myc expression and inhibiting melanoma tumor growth in mouse models. Their preclinical studies underscore the importance of further investigating nanoparticle treatment options for chemo-resistant melanoma.

Introduction

The field of targeted therapeutic strategies in melanoma is entering exciting times. Malignant melanoma is the deadliest form of skin cancer and has for decades represented a paradigm for chemo-resistance. Current therapeutic options are poor and no new US Food and Drug Administration (FDA)-approved drugs have emerged in recent years. Current melanoma treatment mainstays, such as the alkylating agent, dacarbazine, and the immune-stimulating agent, IL-2, are plagued by lack of clinical benefit in the majority of patients and numerous side effects. However, highly encouraging results have been reported within the past fifteen months which have renewed hope that new FDA-approved drugs will be forthcoming. The phase I trial of the RAF inhibitor, RG7204/PLX4032 (Plexxikon, Inc.), found that approximately 75% of mutant B-RAF melanoma patients displayed responses by RECIST criteria (Flaherty *et al.*, 2009). Additionally, a phase III study of Ipilimumab (Bristol-Myers Squibb), an anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) drug, improved overall median survival for metastatic melanoma patients that had received previous treatment (Hodi *et al.*, 2010). While the outlook appears promising, some major difficulties still remain. For example, the majority of patients who initially responded to RG7204 have subsequently relapsed, raising concerns about acquired/secondary resistance. Also, despite the improved median survival data for Ipilimumab, the response rate was only 11%. Hence, the search for additional targeted therapies is urgently needed.

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In this issue, an article from Chen *et al.* focuses on targeting c-Myc, a proto-oncogene that is well-studied in cancer. c-Myc is a basic helix-loop-helix leucine zipper transcription factor that is highly expressed in melanoma (Ross and Wilson, 1998). Importantly for the current study, c-Myc depletion in melanoma cells induces cell cycle arrest and a senescence-like phenotype (Zhuang *et al.*, 2008). Conversely, over-expression of c-Myc in normal melanocytes inhibits mutant B-RAF-induced senescence (Zhuang *et al.*, 2008). These phenotypes may result from alterations in the expression of several c-Myc transcriptional target genes which encode rate-limiting enzymes for dNTP metabolism (Mannava *et al.*, 2008).

Nanoparticle-based melanoma treatment strategies

Chen *et al.* used a c-Myc siRNA depletion strategy combined with a unique liposomal nanoparticle formulation to increase siRNA stability and melanoma cell delivery. They developed nanoparticles formed from a DSAA carrier lipid, instead of the previously utilized DOTAP liposome particles, which were specifically targeted to melanoma cell sigma receptors via an anisamide ligand (DSAA AA+). Compared to DOTAP AA+ nanoparticles, the DSAA AA+ particles containing c-Myc siRNA evidenced higher melanoma cell delivery and c-Myc reduction *in vivo*. In addition, dose-dependent tumor growth inhibition was seen after intravenous administration of DSAA AA+ c-Myc siRNA particles in the syngeneic B16F10 mouse melanoma tumor model and in a human melanoma xenograft system. Perhaps the most striking finding was the complete growth inhibition of B16F10 mouse melanoma tumors when the DSAA AA+ c-Myc siRNA particles were administered in combination with paclitaxel chemotherapy. Interestingly, the biological properties of the DSAA carrier lipid itself seem to add an additional dimension to the potency of this melanoma treatment strategy. Empty DSAA nanoparticles were shown to increase ROS levels, decrease expression of the pro-survival protein Bcl-2, and increase apoptosis in B16F10 cells. Importantly, all treatments with DSAA nanoparticles were demonstrated to have low immunotoxicity. Taken together, these results highlight the potential of utilizing DSAA AA+ cationic nanoparticles to target gene expression as a new melanoma treatment option. These results, along with the possible chemotherapeutic drug combination avenues it opens, may one day offer real hope to advanced melanoma patients, especially those who may have acquired resistance to other treatments.

Chen and colleagues are not the first to utilize nanoparticles as a siRNA delivery vehicle to target melanoma tumors in preclinical assays. Tran *et al.* utilized topical applications of liposomal nanoparticles combined with ultrasound to promote B-RAF^{V600E}-selective and Akt3 siRNAs to penetrate into the skin microenvironment and inhibit the growth of mutant B-RAF melanoma cells in 3-D *in vitro* and xenograft assays (Tran *et al.*, 2008). These studies utilized DOTAP/DOPE/DSPE-PEG(2000)-formulated nanoparticles which may lack the ROS-generating effects observed with the DSAA-formulated nanoparticles used in the Chen *et al.* experiments. A separate study systemically delivered protease-activated receptor-1 siRNA incorporated into neutral DOPC liposomal nanoparticles (Villares *et al.*, 2008). Importantly, protease-activated receptor-1 siRNA-containing liposomes decreased growth and metastasis of human melanoma cells in nude mice.

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Non-liposomal nanoparticles have also been utilized of late as delivery systems for siRNAs and pharmacologic inhibitors into primary and metastatic melanoma tumors. Similar to the Chen *et al.* study in this issue, Zamora-avila and colleagues used the B16F10 mouse melanoma tumor model but instead focused on the treatment of melanoma lung metastases. They formulated Wilm's tumor gene 1 siRNA-polyethylenimine nanoparticles and introduced them into the murine lung via an aerosol-based delivery system. Strikingly, these nanoparticles allowed for decreased lung tumor burden and increased survival without lung tissue damage or acute inflammatory response (Zamora-Avila *et al.*, 2009). Yet another study expanded the scope of the use of nanoparticles to include pharmacological drug delivery with particular focus on improvement of sustained and targeted drug release. Basu *et al.* derived nanoparticles composed of a hexadentate-polylactic acid-glycolic acid polymer conjugated to the MEK pharmacologic inhibitor, PD98059. This formulation demonstrated increased inhibition of melanoma cell proliferation both *in vitro* and *in vivo* compared to traditional drug delivery methods (Basu *et al.*, 2009). In addition, PD98059-conjugated nanoparticles could synergize with cisplatin treatment to demonstrate enhanced anti-tumor activity in the B16F10 mouse melanoma model.

Concluding remarks

Given the scarcity of effective treatment options available to patients with advanced melanoma, the dawn of nanoparticle delivery systems could not come at a better time. Future studies in the field should focus on potential combinations of siRNA- and chemotherapeutic/pharmacological inhibitor-contained nanoparticles since both formulations have been shown to increase delivery and effects. In addition, even though syngeneic mouse models such as B16F10 are highly important in the development of cancer therapies, it would be advantageous to extend these studies into models more relevant to human melanoma progression. Specifically, the recent conditional B-RAF^{V600E} / PTEN-deficient metastatic melanoma mouse is an ideal disease model for preclinical studies of these nanoparticles (Dankort *et al.*, 2009). Additional studies with human melanoma xenograft mice, which allow for representation of a wide range of melanoma genetic backgrounds and staging, would also be of value.

In conclusion, increasing evidence within the past several years has revealed that nanoparticles have the potential to improve the efficacy of current treatments and/or to open doors to strategies such as siRNA targeting which have been plagued with problems. The work of Chen *et al.* extends this area of research and finds that targeting melanoma tumor cells with c-Myc siRNA packaged within the new DSAA nanoparticle, alone or in combination with chemotherapy, can drastically inhibit melanoma tumor growth in several systems. These findings offer new hope for the future development of a potent and efficacious treatment strategy for advanced melanoma.

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Clinical implications

1. Malignant melanoma is a paradigm for chemo-resistance.
2. Nanoparticle delivery of siRNAs represents a selective approach to target any expressed mRNA in melanoma cells.
3. c-Myc expression is elevated in melanoma and targeting c-Myc shows promise in *in vivo* preclinical assays.