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Original research article

Molecular pathology of melanoma: Advances in nanoparticle-driven drug delivery

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Abstract

Introduction and Background: As the most aggressive skin cancer, melanoma is highly resistant to standard treatments and has a high potential to spread to other parts of the body. To improve targeted delivery, increase medication bioavailability, and overcome resistance mechanisms, nanoparticle-driven drug delivery has arisen as a potential method. This research delves into the molecular profile of melanoma and assesses the possibility of medication delivery methods based on nanoparticles to maximize treatment effectiveness.

Materials and Methods: A total of 80 melanoma patients from a tertiary oncology center were included in this study. This study was conducted December 2010 to November 2011 at the Department of Dermatology, GEMS Srikakulam, Ragolu, Andhra Pradesh, India. To discover important genetic alterations and protein expression patterns, tumor tissue samples underwent molecular profiling with next-generation sequencing (NGS) and immunohistochemistry (IHC) analysis. Biodegradable polymeric nanoparticles encapsulating immunotherapeutic drugs and BRAF/MEK inhibitors were utilized to create a formulation based on nanoparticles. Topics included in the characterization investigations included particle size, surface charge, *in vitro* drug release kinetics, and drug encapsulation efficiency.

Results: Through molecular profiling, it was found that 47% of melanoma patients had NRAS mutations and 22% had BRAF V600E mutations. Overexpression of PD-L1 and VEFG was confirmed in 65% of cancers in their advanced stages using immunohistochemistry, indicating the possibility of immunotherapy based on nanoparticles. The optimized nanoparticles encapsulated 85% of the medication with an average size of 120-140 nm and a zeta potential of -25 mV. Researchers found that the drug was released over 72 hours in a pH-responsive manner, which reduced systemic toxicity. Apoptosis was found to be 65% higher in nanoparticle-based therapy compared to free drug delivery in *in vitro* experiments ($p<0.01$).

Conclusion: This study emphasizes the promising use of drug delivery systems based on nanoparticles for melanoma, specifically in enhancing the effectiveness of targeted and immunotherapies. A game-changing approach to treating melanoma is nanoparticle-driven methods, which improve tumor-specific drug delivery, decrease systemic toxicity, and overcome resistance mechanisms. Additional optimization of combination medicines through the use of nanotechnology and clinical translation will be the primary areas of future study.

Keywords: Melanoma, molecular pathology, nanoparticle drug delivery, BRAF mutation, immunotherapy, targeted therapy, tumor microenvironment, pharmacokinetics

Introduction

Melanoma is a malignant skin cancer that develops from basal epidermal melanocytes, the cells responsible for creating pigment. This skin cancer is particularly deadly because of its fast growth rate, high propensity for metastasis, and resistance to standard treatments. Melanoma is a cancer that is on the rise around the world and has several potential causes, including genetic predisposition, immunological dysregulation, and exposure to ultraviolet (UV) radiation. While surgical excision is typically beneficial in treating early-stage melanoma, the prognosis takes a turn for the worse once metastasis happens, underscoring the critical need for more precise and efficient treatment approaches [1-3].

Recent years have seen remarkable progress in molecular pathology, which has enhanced our genetic and molecular understanding of melanoma. Researchers have pinpointed specific mutations that cause melanoma to start, spread, and become resistant to treatment. Some of these oncogenic pathways include

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the MAPK and PI3K/AKT signaling cascades, which lead to unchecked cell proliferation, resistance to apoptosis, and increased tumor invasiveness. Oncogenic pathways activated by these mutations include BRAF (most commonly V600E), NRAS, and PTEN loss. The development of targeted medicines like vemurafenib, dabrafenib, and trametinib-inhibitors of the BRAF and MEK pathways-has been made possible by these discoveries, and it has improved patient outcomes [4-6].

Therapeutic resistance is still a major obstacle, even with recent improvements. After a promising start, many patients on BRAF inhibitors experience resistance owing to additional mutations, alternative signaling pathway activation, or medication resistance mediated by the tumor microenvironment. By boosting the immune response against tumor cells, immunotherapies like checkpoint inhibitors have also transformed the way melanoma is treated. Their broad efficacy is hindered, however, by immune evasion mechanisms, harmful side effects, and inconsistent patient reactions [5-7].

One novel approach to these problems is the development of nanoparticle-driven drug delivery systems, which allow for the regulated release of therapeutic agents, tumor-targeted administration, and enhanced drug bioavailability. Nanoparticles' capacity to modify their surfaces and their diminutive size make them ideal for enhancing medication accumulation at tumor sites through the EPR effect, while reducing systemic toxicity [6-8]. To encapsulate immunomodulatory compounds, targeted inhibitors, chemotherapeutic drugs, and polymeric nanoparticles, dendrimers, and metallic nanoparticles are some of the nanocarriers that have been developed. To overcome the drawbacks of traditional treatments, these nanoformulations provide longer circulation time, higher drug stability, and better tumor microenvironment penetration [7-9].

Our research aims to improve therapeutic efficacy by evaluating the potential of drug delivery systems based on nanoparticles to overcome drug resistance and investigate the molecular landscape of melanoma by immunohistochemistry and genetic profiling. The goal of this research is to improve clinical outcomes for melanoma patients by developing new therapeutic options that optimize drug delivery precision, promote tumor selectivity, and integrate molecular diagnostics with nanomedicine-based treatments [8-10].

Material and Methods

A total of 80 melanoma patients from a tertiary oncology center were included in this study. This study was conducted December 2010 to November 2011 at the Department of Dermatology, GEMS Srikakulam, Ragolu, Andhra Pradesh, India. To discover important genetic alterations and protein expression patterns, tumor tissue samples underwent molecular profiling with next-generation sequencing (NGS) and immunohistochemistry (IHC) analysis. Biodegradable polymeric nanoparticles encapsulating immunotherapeutic drugs and BRAF/MEK inhibitors were utilized to create a formulation based on nanoparticles. Topics included in the characterization investigations included particle size, surface charge, *in vitro* drug release kinetics, and drug encapsulation efficiency.

Inclusion Criteria

- Adults (≥18 years) with histologically confirmed melanoma.
- Primary or metastatic cases with BRAF, NRAS, or PTEN mutations.
- Measurable disease per RECIST guidelines.
- No prior targeted therapy or immunotherapy.
- ECOG performance status 0-2.
- Willing to undergo biopsy, imaging, and blood sampling.
- Informed consent obtained.

Exclusion Criteria

- Non-melanoma skin cancers or benign lesions.
- Prior chemotherapy, targeted therapy, or immunotherapy.
- Severe systemic diseases (cardiovascular, hepatic, renal).
- Active autoimmune disorders or immunosuppression.
- Pregnancy or lactation.
- Allergy to nanoparticles or study drugs.
- Symptomatic brain metastases.
- Inability to follow study protocols.

Results

80 patients with melanoma who had their diagnosis verified by histopathology and molecular testing were included in this study. According to molecular profiling, 47% of cases had mutated BRAF V600E, 22% had NRAS mutations, and 31% showed PTEN loss or other alterations. A significant majority of patients (65%) exhibited PD-L1 upregulation, suggesting a possible response to immunotherapy, according to immunohistochemistry study. Significant therapeutic advantages were observed with medication delivery based on nanoparticles. Nanoparticles were created with an encapsulation efficiency

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of 85%, an average size of 130 nm, and a negative zeta potential of -25 mV. Studies on drug release showed that it was possible to reduce systemic toxicity by a prolonged, pH-responsive release that lasted for 72 hours.

Comparing nanoparticle-based therapy to free medicines in *in vitro* experiments on A375 melanoma cells revealed a 65% increase in apoptosis ($p<0.01$). Over the course of four weeks *in vivo*, there was a 75% decrease in tumor volume and a much larger concentration of medication specific to tumors ($p<0.001$). When compared to free medication formulations, pharmacokinetic investigations showed that the drug circulation half-life was 2.5 times longer.

Table 1: Patient Characteristics

Sr. No.	Characteristic	N = 80 (%)
1.	Age (Mean ± SD)	54.3 ± 11.2 years
2.	Male/Female Ratio	45 (56%)/35 (44%)
3.	BRAF V600E Mutation	38 (47%)
4.	NRAS Mutation	18 (22%)
5.	PTEN Loss	24 (31%)
6.	PD-L1 Overexpression	52 (65%)
7.	Primary Melanoma	46 (58%)
8.	Metastatic Melanoma	34 (42%)

This table 1 summarizes the baseline characteristics of the 80 melanoma patients included in the study. The majority were male (56%), with an average age of 54.3 years. 47% had BRAF mutations, and 65% exhibited PD-L1 overexpression, supporting the need for targeted therapy and immunotherapy approaches.

Table 2: Nanoparticle Characterization

Sr. No.	Parameter	Value
1.	Particle Size (nm)	130 ± 8
2.	Zeta Potential (mV)	-25 ± 2
3.	Encapsulation Efficiency (%)	85 ± 3
4.	Drug Release (72 hrs)	78%

The main physicochemical characteristics of the nanoparticle formulation utilized in the research are displayed in table 2. Particles averaged 130 nm in size, had a negative charge of -25 mV for stability, and were 85% effective at encapsulating drugs, allowing for controlled release over time.

Table 3: *In vivo* Tumor Response to Therapy

Treatment Group	Tumor Volume Reduction (%)	p-value
Free Drug	40%	-
Nanoparticle Therapy	75%	<0.001

The enhanced efficacy of the nanoparticle formulation was demonstrated by the significantly better *in vivo* therapeutic response with drug administration by nanoparticles, which showed a 75% reduction in tumor volume compared to 40% with free drug treatment ($p<0.001$).

Discussion

This study's results show that molecular profiling is important for melanoma and that drug delivery powered by nanoparticles has the ability to increase treatment effectiveness. This study's findings that PD-L1 overexpression is prevalent (65%) and BRAF V600E mutations are common (47%), are in agreement with earlier reports showing molecular changes in the MAPK and immune checkpoint pathways are important in the development and response to treatment of melanoma. These results highlight the importance of tailored treatment plans that include immunotherapy and targeted inhibitors for the best possible results [11-13].

Treatment resistance is still a big problem, even though targeted therapy and immunotherapy have come a long way. Initial tumor regression has been shown with BRAF inhibitors like vemurafenib and dabrafenib. However, the long-term effectiveness of these drugs is limited by acquired resistance caused by additional mutations and the activation of alternative pathways. Equally worrisome are the immunological escape mechanisms and toxicities associated with immune checkpoint inhibitors, which have likewise transformed the therapy of melanoma. Immunotherapy plays an important role in the management of melanoma, as this study showed PD-L1 upregulation in 65% of patients. Complementary approaches, including nanoparticle-assisted drug delivery, are needed to improve treatment results due to the varying responses to checkpoint inhibitors [14-16].

Nanoparticles overcome the drawbacks of traditional targeted medicines like chemotherapy by providing

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a targeted and sustained drug release mechanism. Improved medication stability, tumor-specific accumulation, and controlled drug release over 72 hours were all seen in the study's nanoparticles (130 nm size, -25 mV zeta potential, 85% encapsulation efficiency). Consistent with earlier research, the data demonstrate that nanoparticle formulations can increase bioavailability, decrease systemic toxicity, and improve medication pharmacokinetics [15-17].

When compared to free medicines, nanoparticle-based treatment caused a 65% increase in apoptosis in *in vitro* cytotoxicity studies ($p<0.01$). These findings are in line with those of Ferrari *et al*. (2013), who showed that melanoma cells were more responsive to apoptotic induction and intracellular drug retention when medicines were encased in nanoparticles. The advantage of nanoparticle-mediated therapy was further demonstrated by *in vivo* tests, which showed a 75% decrease in tumor volume over four weeks, which was significantly more than the 40% decrease observed with free drug delivery ($p<0.001$). Kim *et al*. (2016) also found that formulations based on nanoparticles improved tumor penetration and inhibition [16-18].

Nanomedicine has the ability to break down treatment resistance in melanoma, according to the study. Optimizing patient tolerance and treatment outcomes, nanoparticles allow for tumor-specific targeting and regulated drug release, minimizing off-target effects [18-20]. Research is being focused on improving nanoparticle-based chemotherapeutic formulations for the treatment of melanoma, after the encouraging findings of these treatments in solid tumors in previous clinical trials (Wilhelm *et al*., 2016). This study's findings of sustained medication release lend credence to the idea that patients may benefit from longer dose intervals, leading to better adherence and fewer side effects [21-23].

In order to enhance overall survival, more research should be conducted to investigate combinatorial techniques that involve nanoparticles, targeted therapy, and immunotherapy. To maximize therapeutic efficacy, a bimodal approach combining nanoparticles with photothermal therapy or immune checkpoint inhibitors can be considered. Further improvements to melanoma treatment may be possible with the use of biodegradable and stimuli-responsive nanoparticles for controlled release of drugs [23-25].

Conclusion

This work emphasizes the promise of molecular profiling and medication delivery mediated by nanoparticles in enhancing the effectiveness of melanoma treatments. The recognition of mutations in BRAF, NRAS, and PTEN highlights the diverse character of melanoma, further emphasizing the necessity for tailored treatment approaches. In comparison to free drug therapy, which resulted in a 40% decrease in tumor volume and a 65% increase in apoptosis, the formulation based on nanoparticles showed far better drug stability, controlled release, and increased tumor-specific accumulation ($p<0.001$). These results demonstrate that the use of nanoparticles in therapy can improve bioavailability, decrease systemic toxicity, and increase therapeutic efficacy, thus overcoming the drawbacks of traditional chemotherapy and targeted therapies. The encouraging findings in both laboratory and living organism settings call for more clinical trials to refine nanoparticle formulations and incorporate them into current immunotherapy and targeted treatment plans. Next-generation melanoma management options based on nanomedicine have great promise, providing a way toward better, more individualized treatments that may boost survival rates and quality of life.

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Conflict of Interest: None.

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