European Journal of Pharmaceutical Sciences 187 (2023) 106476

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

A comprehensive review on novel targeted therapy methods and nanotechnology-based gene delivery systems in melanoma

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ARTICLE INFO

Keywords:
Melanoma
Nanomedicine
Gene delivery
Targeted therapy
Nanovectors

ABSTRACT

Melanoma, a malignant form of skin cancer, has been swiftly increasing in recent years. Although there have been significant advancements in clinical treatment underlying a well-understanding of melanoma-susceptible genes and the molecular basis of melanoma pathogenesis, the permanency of response to therapy is frequently constrained by the emergence of acquired resistance and systemic toxicity. Conventional therapies, including surgical resection, chemotherapy, radiotherapy, and immunotherapy, have already been used to treat melanoma and are dependent on the cancer stage. Nevertheless, ineffective side effects and the heterogeneity of tumors pose major obstacles to the therapeutic treatment of malignant melanoma through such strategies. In light of this, advanced therapies including nucleic acid therapies (ncRNA, aptamers), suicide gene therapies, and gene therapy using tumor suppressor genes, have lately gained immense attention in the field of cancer treatment. Furthermore, nanomedicine and targeted therapy based on gene editing tools have been applied to the treatment of melanoma as potential cancer treatment approaches nowadays. Indeed, nanovectors enable delivery of the therapeutic agents into the tumor sites by passive or active targeting, improving therapeutic efficiency and minimizing adverse effects. Accordingly, in this review, we summarized the recent findings related to novel targeted therapy methods as well as nanotechnology-based gene systems in melanoma. We also discussed current issues along with potential directions for future research, paving the way for the next-generation of melanoma treatments.

1. Introduction

Melanoma, the deadliest form of skin cancer with an increasing incidence rate worldwide, develops from melanocytes, which are skin pigment cells (Garbe and Leiter, 2009). It most typically generates from epidermal melanocytes, however, initial tumors can occur elsewhere (Uong and Zon, 2010). Malignant melanoma of the skin accounts for about 1% of all malignant skin tumors. The stage of melanoma progression, like other malignancies, predicts the success of treatment. After surgical resection of early-stage melanomas (thin tumors), patients have a 97 percent 5-year survival rate (Balch et al., 2009). Advanced melanoma patients, on the other hand, who have metastases in regional lymph nodes or other organs, have a 5-year survival rate of fewer than 10%. Patients with any other systemic metastases have the worst prognosis, with a 1-year survival rate of 41% (Balch et al., 2001). As a result, the approaches that have already been used to treat melanoma are dependent on the stage of cancer and include the following: 1. Surgery, 2. Radiation therapy, 3. Immunotherapy (Heo et al., 2016). The

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https://doi.org/10.1016/j.ejps.2023.106476
Received 18 March 2023; Received in revised form 17 May 2023; Accepted 22 May 2023
Available online 24 May 2023

severe problems encountered in the medical management of melanoma patients including the damage caused to other tissues which affects the quality of the patients' life whether treated or not, have prompted extensive efforts to elucidate the molecular pathogenesis of malignant melanoma in the hope of finding more effective treatment options.

In the past decade, scientists have discovered genetic changes that lead to the progression of melanomas and even classify this malignancy into distinct groups. Along with the better understanding of the genetic basis of this disease and the development of nanomedicine and therapy based on gene editing tools, there is a promising outlook for the treatment of melanoma.

Therefore, this review seeks to outline the molecular basis of melanoma pathogenesis and recent achievements in its conventional and advanced therapies and gene delivery systems.

2. Molecular mechanisms of melanoma

The molecular mechanism of melanoma has long been under consideration due to the importance of understanding it in providing clinical guidance. Many studies have reported various genetic and environmental factors in the development of melanoma. Family history is seen in 5–10% of melanoma cases (Florell et al., 2005). Previously, penetration of melanoma susceptible genes has been reported from low to high (Barrett et al., 2015). Furthermore, a combination of melanoma-predisposing genes interacting with other genes or environmental factors might result in melanoma pathway activation (Fargnoli et al., 2010). Here we briefly review some of the most important melanoma susceptibility genes known with high and intermediate penetrance (Table 1).

Depending on geographic region of the families, almost 20–40% of hereditary cases of melanoma are caused by mutations in cyclin-dependent kinase inhibitor 2A (*CDKN2A*) gene, which is located on chromosome 9p21 (Goldstein et al., 2007; Begg et al., 2005; Bishop et al., 2002). p16 (INK4A/inhibitor of kinase 4A) and p14 (ARF/alternate reading frame) are tumor suppressor genes encoded by the *CDKN2A*, which both involve in regulation of cell cycle. By inhibiting cyclin-dependent kinase 4 (CDK4) and cyclin-dependent kinase 6 (CDK6) activities, the p16 inhibits the phosphorylation of retinoblastoma protein (pRb) and modulates the transfer of G1 to S phase, which are involved in aging and regulation of damaged cells. p14 interacts with MDM2 and performs through p53 pathway to stop cell cycle or favoring apoptosis (Chudnovsky et al., 2005; Goldstein et al., 2006). According to the population-based analysis, the penetration of *CDKN2A* mutation has been found to vary between different geographical areas (Bishop et al., 2002; L.G. Aoude et al., 2015).

Mutation in *CDK4* on chromosome 12q14 has been detected in some melanoma families worldwide (Bottillo et al., 2018). Codon 24 of exon 2 includes all pathogenic mutations of *CDK4*, which lead to disruption of the cell cycle in the G1 phase. *CDK4* and *CDKN2A* mutated families indicate indistinguishable phenotype due to both mutations involve same pathway (Puntervoll et al., 2013).

BRCA1-associated protein-1 (*BAP1*), having a chromosomal location 3p21, acts as a tumor suppressor, regulating differentiation of melanocytes and DNA damage repair. Independent studies have reported germ line mutations of *BAP1* gene in distinct syndromes that melanoma is considered as part of the phenotype of those syndromes (Rossi et al., 2019). The diversity of cancers in *BAP1*-mutated families in addition to the importance of the *BAP1* gene and its variable penetrance, also emphasizes the roles of environmental factors or modifier genes (L.G. Aoude et al., 2015).

Telomerase reverse-transcriptase gene (*TERT*) encodes a catalytic subunit of telomerase, which plays an important role in cancer including melanoma by maintaining the length of telomere and preventing cell aging (Vinagre et al., 2013). Recently, a rare but highly penetrant germline mutation has been found in the promoter of *TERT* gene (on chromosome 5p15) in families with early-onset melanoma (Horn et al., 2013).

In human cells, the shelterin complex consisting of six telomere-specific proteins (POT1, TRF1, TRF2, RAP1, TIN2 and TPP1) binds along the length of telomere tract to maintain chromosome stability and protect against degradation (Diotti and Loayza, 2011). According to different studies, several mutations has been detected in protection of telomeres 1 gene (*POT1*, on chromosome 7q31), encoding an imperative member of the shelterin complex proteins in melanoma patients (Muller et al., 2018). Most loss-of-function germline mutations in *POT1* associated with melanoma occur in the oligonucleotide-/oligosaccharide-binding (OB) region of the gene, leading to insufficient telomeres coverage by the shelterin complex and abnormal telomeric length (Robles-Espinoza et al., 2014; Shi et al., 2014).

Recently, mutations in other shelterin complex members including adrenocortical dysplasia protein homolog (*ACD*) and telomeric repeat binding factor 2 interacting protein (*TERF2IP*) genes have been demonstrated to be effective in predisposing to melanoma. ACD contains a domain of POT1 protein binding and hence together with POT1 mediated the interaction of the shelter complex with TERT. Furthermore, TERF2IP associates repression of homologous repair of double strand chromosome breaks and regulation of the NF-κB signaling pathway (Read et al., 2016; L.G. Aoude et al., 2015). Generally, pathogenic ACD and TERF2IP have been associated to early melanoma identification and an elevated risk of a variety of primary melanomas (Pastorino et al., 2020).

Interestingly, moderate-penetration melanoma-prone genes identified to date are associated with natural changes in skin pigmentation (Read et al., 2016). Melanocortin 1 Receptor (*MC1R*), as a moderate risk gene, is located on 16q24 and encodes the α melanocyte-stimulating hormone (α-MSH) receptor 1. *MC1R* is a highly polymorphic gene

Table 1
Overview the most important genes involved in melanoma susceptibility.

Gene Penetrance	Gene	Location on chromosome	Protein function	Mutation prevalence
High-Penetrance	Cyclin dependent kinase inhibitor 2A (*CDKN2A*)	9p21	Cell cycle regulator	20–40%
	Cyclin dependent kinase 4 (*CDK4*)	12q14	Cell cycle regulator	<1%
	BRCA1 associated protein 1 (*BAP1*)	3p21	Transcriptional regulation and DNA damage repair	<1%
	Telomerase reverse transcriptase (*TERT*)	5p15	Telomere elongation	<1%
	Protection of telomeres 1 (*POT1*)	7q31	Telomere maintenance	<1%
	adrenocortical dysplasia protein homolog (*ACD*)	16q22	Regulation telomere processing and stability	<1%
	TERF2 interacting protein (*TERF2IP*)	16q23	Regulation telomere processing and stability	<1%
Intermediate-Penetrance	Melanocortin 1 Receptor (*MC1R*)	16q24	Melanin production	70–90%
	Microphthalmia-associated transcription factor (*MIFT*)	3p13	Melanocyte development and differentiation	1–5%
	Solute carrier family 45, member 2 (*SLC45A2*)	5p13	Melanin production	NA

NA= Not Applicable.

involved in triggering DNA damage repair and regulating both hair and skin pigmentation (Zocchi et al., 2021). Phenotypes including red hair, freckling, UV sensitivity, and melanoma risk are commonly associated with *MC1R* variants (Chen et al., 2019).

MITF, or Microphthalmia-associated transcription factor, is a transcription factor considered as a regulatory key in cell pigmentation and melanocytes development and differentiation (Hartman and Czyz, 2015). p.E318K (rs149617956) is a common and gain-of-function variant of *MITF* which causes increases melanoma risk and renal cell carcinoma development by impaired protein accumulation and stimulation of hypoxia-induced factor 1A (HIF1A), respectively (Bertolotto et al., 2011; Yokoyama et al., 2011).

The *SLC45A2* gene (family of 45 solutes, member 2) is found on the short arm of chromosome 5 (5p13.2), which encodes a transporter protein involved in melanin synthesis and is one of the medium-penetrance genes associated with cutaneous melanoma. In various cases, the ancestral variant rs16891982 (p.L374F), which was associated with olive and dark skin, has shown a protective effect against melanoma and has retained this role for light-skinned people (Read et al., 2016).

3. Conventional therapies

Various therapeutic approaches are available for patients with melanoma. The patient's underlying medical problems and the thickness of the primary melanoma, the stage of the melanoma, distant metastases, unique genetic mutations in melanoma cells, and the rate of melanoma growth, all influence the therapeutic strategy (Davis et al., 2019). Surgical resection, chemotherapy, radiotherapy, immunotherapy, and targeted therapy are some of the current melanoma treatment options (Domingues et al., 2018). The main therapy for melanoma is the surgical removal of the primary melanoma on the skin, often known as excision (Rutkowski et al., 2010). While surgery is the most common treatment for initial stage melanoma, more advanced melanoma is far more difficult to cure due to the inefficacy of standard cancer medicines like chemotherapy (Guan et al., 2021; Mohammadpour et al., 2019). Next, melanoma therapeutic approaches are briefly described (Fig. 1).

3.1. Excisional surgery

After diagnostic confirmation of melanoma, based on its pathologic stage, the cancerous lesion along with a margin of surrounding healthy tissue should be removed (Friedman et al., 2021). The surgical methods vary depending on the clinicopathologic characteristics of the tumor. In early-stage melanomas (stages I and II), the main goal of surgical removal, as a gold standard, is to achieve local control of the melanoma and increase the patient's overall survival (Rutkowski et al., 2010).

In patients with stage III melanoma, where cancerous cells spread to the lymph nodes, multiple treatment options such as a lymphadenectomy or surgery to remove the lymph nodes can be used (Atkins et al., 2021). Melanoma has already spread to distant lymph nodes or other body regions, such as the brain, spinal cord, lungs, liver, bone, or gastrointestinal (GI) tract, in the most aggressive stage (Stage IV) (Leung et al., 2012). Surgery for this advanced melanoma is frequently combined with immunotherapy or targeted therapy (Tyrell et al., 2017).

3.2. Radiotherapy

Most people with cutaneous melanoma do not require radiation therapy, which may be beneficial in limited cases (Strojan, 2010). For example, it can be a choice to treat very early melanoma in some cases that surgery cannot be used or in some uncommon types of melanoma (desmoplastic melanoma) after surgery (Foote et al., 2008). In some cases, radiotherapy may be given after surgical removal of the tumor-bearing lymph node to reduce the chance of cancer recurrence. In some patients whose melanoma comes back after surgery, radiation therapy can be helpful to either the skin or lymph nodes. Sometimes radiation therapy can be used as palliative therapy to reduce tumor growth and help control some of the symptoms, or in metastatic patients that have pain and their vertebral column stability is threatened (Garbe et al., 2008).

The type of radiation is based on the goal of treatment and the place

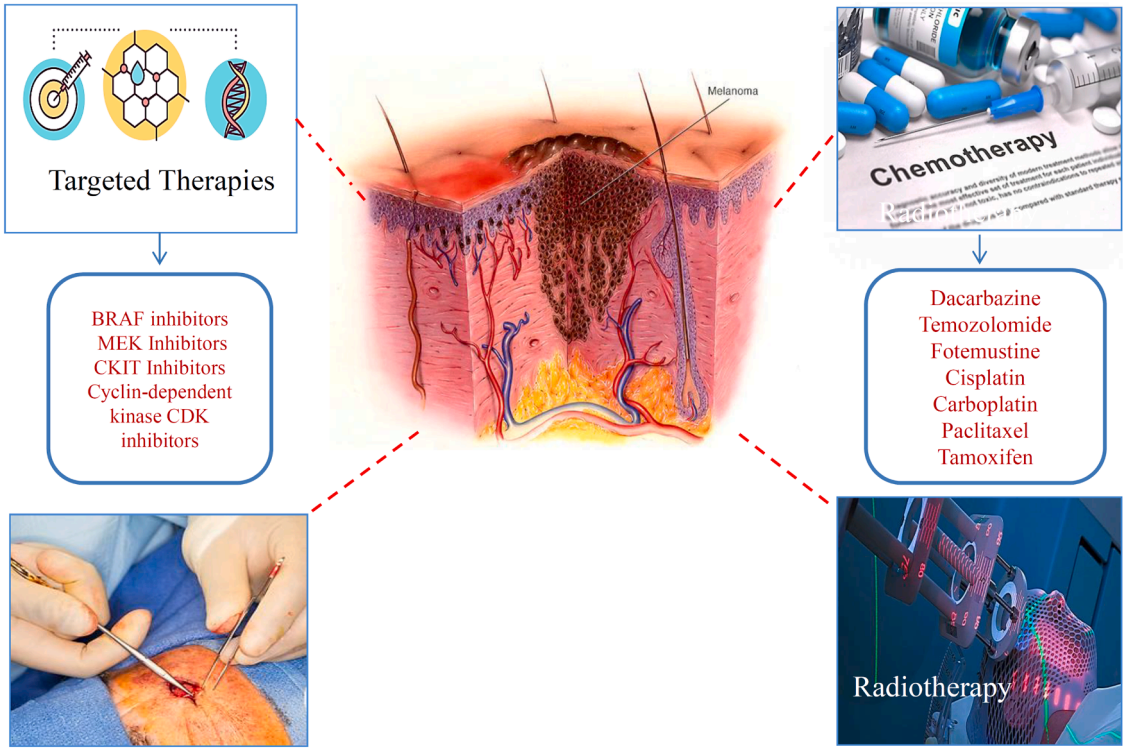

Fig. 1. Schematic representation of conventional therapies for melanoma.

of melanoma (Borzillo and Muto, 2021). Radiotherapy destroys cancer cell's DNA through high-energy radiation (Baskar et al., 2014). The melanoma cells with high proliferative power, low cell differentiation, and their enzymatic system quickly repair the damage caused by low-dose radiotherapy, so they are relatively radioresistant (Carlos--Reyes et al., 2021). Therefore, radiotherapy and its dose for a patient should be made after various evaluations (Oliveira Pinho et al., 2019). Most systemic medications have limited penetration into the CNS, so radiation therapy (whole-brain irradiation and/or stereotactic radiosurgery) is especially beneficial in patients with CNS metastases (Bhatia et al., 2009).

3.3. Chemotherapy

In advanced melanoma, surgery and radiotherapy are insufficient, and patients need to be treated with chemotherapeutic agents. Some decades ago (1970s), the only single-agent approved for the treatment of melanoma was the alkylating agent dacarbazine (DTIC, dimethyltriazeno imidazole carboxamide) (Eggermont and Kirkwood, 2004). Dacarbazine, like other chemotherapeutic drugs, is nonselective, and in addition to killing cancer cells it may cause damage to healthy tissue (Kuryk et al., 2020). On the other hand, clinical trials show that this drug has moderate efficacy against tumors. Nevertheless, dacarbazine is still one of the main treatments in chemotherapy for metastatic melanoma (Mishra et al., 2018).

Several studies have been performed on the adjunct use of dacarbazine with chemotherapy, immunotherapy, natural health products, and natural compounds (Sood et al., 2021).

The analog of dacarbazine, temozolomide (TMZ), is an approved chemotherapeutic agent commonly used for glioblastoma multiforme and reaches the central nervous system (Strobel et al., 2019). Since temozolomide can cross the blood-brain barrier, it can operate in melanoma patients with brain metastases (Quirt et al., 2007).

Different studies have shown that melanoma cells can be resisting against chemotherapeutic drugs by resistance to apoptosis (Soengas and Lowe, 2003). They can reprogram their proliferation process and affects their survival (Wilson and Schuchter, 2016). Several other chemotherapeutic agents have been examined on melanoma progression, shown below.

Fotemustine, a member of the *nitrosourea family*, is a cytotoxic alkylating agent. By adding a chloroethyl group to the guanine base functions in the same way as DTIC and TMZ (Quereux and Dreno, 2011). It isn't affirmed by the FDA for utilization in metastatic melanoma but has appeared to have comparable or marginally higher reaction rates to dacarbazine in stage III trials (Avril et al., 2004).

The platinum drugs, cisplatin, and carboplatin are utilized broadly for metastases treatments. These drugs can alkylate DNA at purine residues (Johnstone et al., 2014). It is guessed that the most component of actuating apoptosis for platinum analogs is by repressing the transcription of RNA (Todd and Lippard, 2009).

Carboplatin was tested in a Phase II trial, which showed a response rate ranging from 10% to 25%, comparable to dacarbazine (Yang and Chapman, 2009).

Paclitaxel, a microtubule-targeting agent, is one of the oldest chemotherapeutic agents. Its function can lead to mitotic arrest and apoptotic cell death (Abal et al., 2003). A phase II clinical trial of paclitaxel (dosage of 250 mg/m2) showed an overall response rate of 12% for four metastatic melanoma patients (Legha et al., 1990).

Tamoxifen, a selective estrogen receptor modulator, is commonly used in HR+ breast cancer patients (Zhou and Liu, 2020). Some metastatic melanoma patients have shown that cytoplasmic estrogen receptors are more highly expressed (Fisher et al., 1976). So, tamoxifen has been used in the investigation for metastatic melanoma treatment (Lens et al., 2003). It has been shown that a combination of dacarbazine with tamoxifen is more effective for metastatic melanoma than dacarbazine alone (Cocconi et al., 1992).

Cannabinoids are a class of compounds derived from Cannabis sativa, with Δ9-tetrahydrocannabinol (THC) the most notable for its high potency and abundance (Amin and Ali, 2019). THC mimics endocannabinoids and leads the activation of two G protein–coupled cannabinoid receptors: CB1 and CB2 (Pertwee, 2006). Cannabinoids display a powerful anticancer effect in various cellular and animal models (Velasco et al., 2007), and they have been largely explored in combination with other anticancer drugs in preclinical studies (Hinz and Ramer, 2022; Guzman et al., 2006). Notably, some studies showed that Cannabinoids exert antiproliferative action on melanoma cells through a complex modulation of signaling pathways and cellular mechanisms which lead to the i) induction of cytotoxic autophagy, ii) cell cycle arrest, iii) inhibition of Akt signaling and iv) activation of the pRb retinoblastoma protein tumor suppressor (Armstrong et al., 2015; Blázquez et al., 2006).

3.4. Targeted therapies

Around 70% of cutaneous melanoma patients have mutations in critical signaling pathway genes. Melanoma cell growth and a malignant phenotype may be linked to specific oncogenic alterations (Franklin et al., 2017).

Targeted therapies include the use of different cancer inhibitors such as BRAF inhibitors(Vemurafenib and Dabrafenib), MEK Inhibitors (Trametinib), CKIT Inhibitors(Imatinib), Cyclin-dependent kinase (CDK) inhibitors(ribociclib, abemaciclib, and palbociclib) (O'Leary et al., 2016).

Compared to traditional chemotherapeutic agents, Vemurafenib is a highly selective mutant BRAF inhibitor that is very effective in patients with metastatic melanoma who have a BRAFV600 mutation (including patients with non V600E mutations). It improves survival rates and elicits responses in 50% of patients (C. Robert et al., 2015; Robert et al., 2014).

Despite vemurafenib's therapeutic efficacy, most patients, and some are not, develop resistance to treatment (Swaika et al., 2014).

Different resistance proteins are known in two classifications (intrinsic and extrinsic), some of them reactivate the MAPK pathway, and the others are outside of MAPK pathway (Sumimoto et al., 2006).

Dabrafenib is a next-generation agent with a mode of action similar to vemurafenib. It's also a selective BRAF mutant inhibitor approved by the FDA in 2013 to treat BRAFV600E-mutated unresectable or metastatic melanomas (Ballantyne and Garnock-Jones, 2013; Livingstone et al., 2014).

Trametinib, a MEK1/MEK2 inhibitor with antitumoral efficacy, is approved for the treatment as monotherapy for BRAFV600 mutant metastatic. MEK, the extracellular signal-regulated kinase downstream of BRAF, is suppressed with the addition of trametinib (Liu and Sheikh, 2014). In a randomized and multicenter study, trametinib and dabrafenib (BRAF-mutant inhibitor) produced persistent objective responses. The FDA (2014) approved the combination to treat metastatic melanomas with BRAF mutations (Flaherty et al., 2012; Niezgoda et al., 2015).

Imatinib, a CKIT inhibitor, has shown to be effective in individuals with metastatic melanoma who have CKIT mutations, with a 30% response rate (Hodi et al., 2013).

Sunitinib, dasatinib, and nilotinib, among other multikinase inhibitors, may be effective in melanoma patients with KIT mutations (Livingstone et al., 2014).

Tumors can now be targeted more effectively and with fewer side effects due to a new class of specific CDK4/6 inhibitors such ribociclib, abemaciclib, and palbociclib (O'Leary et al., 2016). Abemaciclib has also been shown to cause growth regression in melanoma models resistant to vemurafenib, with significant cyclin D1 expression and MAPK pathway reactivation (Yadav et al., 2014).

4. Advanced therapies

4.1. Nucleic acid (ncRNA, aptamers) based therapies

Nucleic acid therapies, as prospective options, have lately garnered attention in the field of cancer treatment. Different nucleic acid therapeutics options have versatile functionalities such as alteration of gene expression and regulation of immune response (Zhou et al., 2020; Kulkarni et al., 2021).

Non-coding RNA (ncRNA) is employed for a class of RNA that does not translate into a protein, which is divided into two groups, small non-coding RNA (sncRNA) and long non-coding RNA (lncRNA), based on size. Up to now various studies have highlighted the involvement of several lncRNAs including PAUPAR, HOTAIR, CANT1, MALAT1, GAS5, BANCR, SNHG7, ANRIL, ZNNT1, SPRY-IT1, UCA1, RMEL3, SAMMSON, etc., in the pathogenesis and metastatic process of different types of melanoma (Wei et al., 2016; Bhan et al., 2017; J. Li et al., 2016; Goedert et al., 2016; Milan-Rois et al., 2021). Promising therapeutic strategies against lncRNAs have been focused on selective knockdown upregulated or downregulated lncRNAs expression as well as some lncRNAs which might be indirectly applied in melanoma. For instance, Shiqiong Xu et al. indicated ANRIL lncRNA silencing using RNA interference (RNAi) methodology could correct INK4a and INK4b defects at the same time and endogenously, afterwards enhanced INK4a and INK4b expression. Moreover, this treatment in melanoma causes to diminish tumor metastatic capacity and tumor formation ability in vitro and in vivo (Xu et al., 2016).

Aptamers are single-stranded oligonucleotides (DNA or RNA) or peptides that fold into a specific target molecule with high affinity and specificity and could be consider as potential therapeutic tools for cancers including melanoma. Accordingly, various studies have examined the effects and efficacy of different aptamers on melanoma (Xiao et al., 2021; Li et al., 2014; Zeng et al., 2018; Wang et al., 2016; Pereira et al., 2018). Some aptamers including multivalent aptamer nanoparticles (X-polymers) have indicated inhibitory effect on melanoma B16 cell growth both in vitro and in vivo (Bai et al., 2020). In a recent preclinical study, an RNA aptamer (GL21.T) has been used against AXL-expressing breast cancer and melanoma cells, which inhibited the tumor via adjusting miR-148b and subsequently silencing of ALCAM and ITGA5 (Quirico et al., 2020). Nakamura et al. demonstrated the growth and liver metastasis of malignant melanoma could be suppress by blocking advanced glycation end products (AGEs) and their receptor (RAGE) system, macrophage infiltration and angiogenesis via RAGE-targeting DNA aptamers (Nakamura et al., 2017). In similar studies, tumor growth was prevented using DNA aptamers, followed by disruption of the AGE/RAGE axis. (Nakamura et al., 2019; Ojima et al., 2014). Furthermore, the use of some aptamers as a drug delivery system in the treatment of melanoma has been reported to be successful (Soldevilla et al., 2016; Li et al., 2019; Lopes-Nunes et al., 2020).

Targeted molecular imaging for specific diagnosis of cancer such as melanoma in tissue imaging and in vivo imaging is considered as one of the applications of aptamers (Sicco et al., 2021), though different uptake behaviors have been indicated in vitro, and in-vivo imaging (Calzada et al., 2017). For instance, due to the overexpression of human matrix metalloprotease-9 (hMMP-9) in cutaneous melanoma, a radiolabeled RNA aptamer, named ^{111}In-DOTA-F3B, has employed for molecular imaging. Results of quantitative biodistribution assessment indicated an increased grade-dependent signal in melanoma tumors as well as accumulation in digestive tract (Kryza et al., 2016).

Circular RNAs (circRNAs), which play regulatory roles in the progression of cancers, are a novel class of covalently closed endogenous RNA molecules (Lux and Bullinger, 2018). Differentially expressed circRNAs and their impact on melanoma have recently been revealed in different studies (Yang et al., 2018; Tang et al., 2021; Ju et al., 2018). According to in vitro, human, and human studies, it seems some circRNAs or their antagonists may be regarded as therapeutic targets in the management and treatment of melanoma, however, further research is awaited to find appropriate strategies for these approach (Zhibing et al., 2020). To give an example, circular RNA hsa_circ_0062270 was upregulated in melanoma cells and could affect cell division cycle protein 45, the host gene of hsa_circ_0062270, at the transcriptional level. Short hairpin RNA (shRNA) targeting hsa_circ_0062270 caused inhibition of invasion, proliferation, viability, and enhancement apoptosis. Additionally, the melanoma tumor growth was restrained in vivo (Hao et al., 2022). CircRNA 0,084,043 was also significantly overexpressed in melanoma and may play a regulatory role for tribbles homolog 2 (TRIB2) through sponging miR-429. When has-circ_0084043 was knocked down, cell proliferation, migration, and invasion could be suppressed, and cell apoptosis could be promoted in vitro and in vivo (Chen et al., 2020). Upregulation of hsa_circ_0025039 was detected in melanoma and knocking down of this circRNA inhibited the tumor growth, invasion, and glucose metabolism. According to the role of hsa_circ_0025039 in regulating CDK4 expression via sponging miR-198, silencing of this circRNA caused the reduction of the tumor volumes and CDK4 expression in a melanoma xenograft model (Bian et al., 2018)

4.2. Immunotherapies

Immunotherapy has been one of the most charming approaches of progress in oncology in the previous decade and changing its outlook. The interaction of the immune system with molecules on the surface of cancer cells is referred to as the molecular mechanism of immunotherapy. The adaptive immune system and cytotoxic lymphocytes T play a crucial role in the immunological response to neoplasms (Lugowska et al., 2018). Immune checkpoints are necessary for a healthy host to prevent autoimmunity and protect healthy tissue during an immune response to infections. On the other hand, tumor cells can use immune checkpoint mechanisms to avoid detection and demolition by the host immune system. Accordingly, immune checkpoint inhibitors intervene at various points in the interaction process between APCs, T-cells, and tumor cells to improve immune system activation and tumor cell targeting because malignant melanoma is immunoreactive cancer, it is a primary target for immunotherapies. Therefore, numerous antibodies for cancer therapy have been approved. The anti-PD-1 medicines (nivolumab, pembrolizumab) (C. Robert et al., 2015) and anti-CTLA-4 antibody ipilimumab (Zimmer et al., 2015) were the first immunotherapies authorized by the FDA for metastatic/unresectable cutaneous melanomas, which account for the vast majority of skin cancer mortality.

Midkine (MDK) is a neurotrofic and developmental factor that plays a role in the regulation of several physiological functions (Tang et al., 2015). Recently, Soengas et colleagues described MDK as a melanoma-secreted factor that induces neolymphangiogenesis and establish pre-metastatic niches (Olmeda et al., 2017). Notably, MDK contributes to an immune evasive microenvironment that defines poor clinical outcome and resistance to immune checkpoint inhibitors. In this regard, genetic manipulation of MDK has been found to sensitize melanoma cells to anti-PD-1/anti-PD-L1 treatment (Cerezo-Wallis et al., 2020).

Furthermore, many therapies have been implemented, including cytokine therapy, vaccinations, combination options, and targeted therapy (Dany et al., 2016). For melanoma with the hotspot mutation in BRAF codon V600, which is present in approximately 50% of patients, a targeted therapy that disrupts the molecular mechanisms essential for cancer cell proliferation and metastasis has been extended (Onitilo and Wittig, 2020; C. Robert et al., 2015). Many trials have proven that the best treatment for these patients is a combination of a BRAF inhibitor and a MEK inhibitor (C. Robert et al., 2015; Robert et al., 2017; Schumacher and Schreiber, 2015). Combination therapy is impressive therapeutic feasibility and could be a combination of coinciding PD1/PD-L1 blockage and the BRAF pathway inhibition (Lugowska et al., 2018). Recent discoveries in genetically modified immunotherapy have

enhanced oncology and broadened the therapeutic options for metastatic melanoma. Talimogene laherparepvec (T-VEC) is a one-of-a-kind oncolytic virus that has been genetically designed to target tumor cells while also boosting the antineoplastic immune response (Greig and Laherparepvec, 2016). T-VEC is a herpes simplex-1 virus that has been genetically engineered. To achieve tumor selectivity, the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene was activated during T-VEC genetic engineering, whereas genes for ICP34.5 and ICP47 were eliminated (Hu et al., 2006). ACT involves collecting lymphocytes from the patient, selecting, growing, and activating the lymphocytes in vitro, and then reinfusing the processed cells back into the patient to induce anti-cancer immune responses. The most commonly used cells in the ACT are peripheral blood lymphocytes (PBL) or tumor-infiltrating lymphocytes (TIL) (Dudley et al., 2008; Wu et al., 2012). The unique ACT method is an injection of isolated and expanded autologous CD4+ T-cells triggered through the melanoma-associated antigen NY-ESO-1 (Hunder et al., 2008).

Furthermore, immune cells that have been genetically modified to represent a tumor-specific receptor can be cultivated and sent back to the patient. The most frequent cells employed in this process are natural killer cells, lymphokine-activated killer cells, cytotoxic T cells, and dendritic cells. Some clinical trials have investigated the effectiveness of this therapy approach in melanoma and other malignancies. Clinical investigations have revealed that ACT effectively treats patients with metastatic melanoma (Dudley et al., 2008; Rosenberg et al., 2008). Adoptive T-cell therapy offers fewer adverse effects than other treatment techniques, such as chemotherapy. In addition, because T cells are cancer-specific, ACT is more tumor-specific and causes minor damage to normal non-cancerous cells. The area of gene therapy is being transformed by gene editing, and ACT is at the forefront of this transformation. To develop off-the-shelf ACT products, gene-editing tools can be employed to re-design the phenotypic of T lymphocytes to improve their anti-tumor effectiveness. Employing homology-directed repair (HDR) donor schemas, remodel endogenous TCRs with tumor-specific TCRs or tumor-reactive chimeric antigen receptors (CARs) T cells (Puig-Saus and Ribas, 2019). Key findings enabled a critical advancement to avoid the requirement to separate and increase the patient's own tumor-reactive T cells that had been genetically modified to target the tumor. The initial breakthrough was finding tumor-associated antigens, similar in patients with various cancer histologies, and can cause a cellular immune response (Coulie et al., 2014). These non-mutated antigens are expressed by antigens produced from improper intronic sequences, common cancer-specific mutations like BRAFV600E and KrasG12D, or alternative open reading frames (Ilyas and Yang, 2015). The non-mutated antigens are classified as lineage-specific antigens or germline antigens. Cancer and normal cells carry lineage-specific antigens, such as melanosomal differentiation antigens (Puig-Saus and Ribas, 2019). The second finding was the detection of receptors that provide tumor reactivity of T cells, paving the way for the capacity to create sophisticated tumor-reactive receptors. This was accomplished by isolating T-cell clones that were reactive to tumor antigens presented by MHC molecules and sequencing their T-cell receptors (TCRs) (Cole et al., 1995). Concurrently, another technique was being pursued: linking monoclonal antibodies with variable domains [single-chain variable fragment (scFv)] selective for antigens expressed on cancer cell surfaces to T-cell signal transduction domains in order to construct tumor-reactive chimeric antigen receptors (CARs) (Eshhar et al., 1993). The third innovation was cloning tumor-reactive TCR and CAR sequences into viral vectors and their use in PBL genetic modification, resulting in new tumor-targeted T cells (Hughes et al., 2005). The essential requirement of viral vectors stemmed from the difficulties of efficiently and persistently expressing foreign transgenes in T lymphocytes, as physical techniques had previously given relatively poor effectual transfection in this cell type. So, various TCRs for common antigens and CARs were found in the years that followed and cloned into retroviral or lentiviral vectors (Morgan et al., 2003; Fesnak et al., 2016). The first clinical study utilized autologous modified T cells to target metastatic melanoma with high expression of MART-1 in the setting of the common HLA allele, HLA-A*02:01 (which is expressed in 40% of the general population). The anti-cancer responses recorded in this experiment comprised perfect responses among fifteen patients. (Morgan et al., 2006). Other clinical studies have also yielded significant clinical responses, including genetically modified T cells targeting MART-1, NY-ESO-1, and other antigens by specific TCRs. Clinical studies designed to target NY-ESO-1-positive malignancies reported 50–61% response rates, 55–66%, and 80% in synovial cell sarcoma, melanoma, and multiple myeloma patients, respectively (Rapoport et al., 2015; Robbins et al., 2011). Given the considerable developments in genetically modified immunotherapy in malignancies, particularly melanoma, the future seems bright and optimistic for more effective therapies.

4.3. Suicide gene therapies

Recently, among different cancer gene therapy approaches, the use of suicide genes is gaining more interest. Suicide gene therapy is based on the inserting a suicide gene into tumor cells which leads to the conversion of different nontoxic compounds into a very toxic compounds and subsequently lead to tumor cell death (Tamura et al., 2021). As a result, the active prodrug exhibits bystander effect via gap junctions or through a variety of other mechanisms, killing both the transfected cancer cells and non-transfected cells (Düzgüneş, 2019).

The two most extensively used suicide genes in cancer therapies are the cytosine deaminase gene (CD) of E. coli, which transforms the nontoxic anti-fungal agent 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU), and the herpes simplex virus thymidine kinase gene (HSV-tk) with ganciclovir (GCV) as prodrug (Gholami, 2017).

The production of viral thymidine kinase subsequent to HSV-tk gene expression, leads to the metabolization of GCV to ganciclovir monophosphate. Cellular kinases then convert mono-phosphorylated GCV into GCV-triphosphate which can inhibit the DNA polymerase function. In addition, the GCV-triphosphate competes with dGTP and incorporates into the DNA strand and causes chain termination and tumor targeted cell death. It was established that inaccurate incorporation of this toxic metabolite into the DNA leads S phase delay, as well as G2-phase arrest by the activation of 3′ exonuclease and post-replicative endonuclease repair mechanisms. As a result of GCV-induced cell cycle arrest, experimental results from recent studies showed that apoptosis and mitochondrial damage has a significant effect in HSV-tk-transduced B16F10 melanoma cell death (Wei et al., 1998; Tomicic et al., 2002; McNeish et al., 2001; Abate-Daga et al., 2010). Due to a low affinity of the human thymidine kinase for ganciclovir, the toxicity related to this drug has limited in humans (Gane et al., 1997). Specifically, a few examples of clinical applications of suicide genes have been reported for melanoma. A significant antitumor effect was initially demonstrated in a B16 melanoma model (Vile and Hart, 1993) and in a xenogeneic melanoma model (Bonnekoh et al., 1996). Klatzmann et al. designed a phase I/II dose-escalation study of HSV-tk type 1 suicide gene therapy for patients with metastatic melanoma using a gamma-retroviral vector as gene delivery platform. In the treated group, the tumor size was moderately affected during ganciclovir treatment compared with untreated tumors. However, tumor size significantly affected and tumor necrosis was identified in nodules injected with the vector in three of eight patients, suggesting a direct toxic effect of ganciclovir triphosphate. The low transfection rate might be the major limited step for the efficacy of this targeting strategy. To overcome these limitations, improving gene transfer methods with the aim of increasing the efficiency of transgene expression should be developed in future studies (Klatzmann et al., 1998).

The limited antitumor effects of HSVtk genes were well documented in several studies in patients after long-term follow-up (Menezes et al., 2018). A phase I clinical study demonstrated the effect of a modified GCV suicide gene in patients with cutaneous or subcutaneous metastatic

malignant melanoma through adenovirus RSV-TK by direct intra-regional injection (NCT00005057).

The anti-tumor effect of direct injection of the *HSV-TK* gene carrying gamma retrovirus particles into the tumor tissue followed by ganciclovir administration in 13 melanoma patients were assessed in a separate trial. Despite some side effects in prodrug treated group, there was no significant responses in injected and non-injected tumors (Singh et al., 2001).

Kucerova et al. have studied the tumor specific effect of the human fat-derived mesenchymal cells which transduced with a gammaretrovirus encoding the CD gene in a mouse model of melanoma. In this study, an inherent property of mesenchymal stem cells to migrate and infiltrate to and within the melanoma tumor mass was used. The injection of the CD-expressing mesenchymal cells shows a significantly tumor progression inhibition after 5FC treatment (Kucerova et al., 2008).

These results and similar studies provide a promising approach in the treatment of melanoma and show signs of clinical benefit of suicide gene therapy-based strategies.

4.4. Gene therapy using tumor suppressor genes

Targeting tumor suppressor gene pathways is an attractive therapeutic approach in cancer. Tumor-suppressor genes have important functions in maintaining genome integrity as well as in regulating cell proliferation, apoptosis, and differentiation. Their loss-of-function mutations are involved in the molecular bases of tumor pathogenesis. Thus, transfer of tumor-suppressor genes directly to cancer cells has been investigated as a therapeutic strategy in both experimental and clinical researches (Wang et al., 2006; Fang and Roth, 2003).

The p53 gene is the most frequently mutated tumor suppressor gene and also, the most common target for replacing defective tumor suppressor genes used in clinical trials (Levine, 1997; Loureiro et al., 2020). Overexpression of wild-type p53 in murine or human melanoma cell lines using adenoviral vector resulted in apoptosis of these cells in vitro as well as inhibition of tumor growth in vivo (Cirielli et al., 1995).

Recently, Dummer et al. assessed the biological activity and safety of intra-tumoral injection of a replication-defective adenoviral expression vector containing wild-type p53 in a phase I dose-escalation study in patients with breast cancer or metastatic form of melanoma with increased p53 protein immune-reactivity in pretreatment tumor biopsies. The results showed minimal toxicity, safety, and feasibility as well as biologically effectiveness of p53 gene therapy by intra-tumoral injection of a replication-defective adenoviral expression vector in patients with either metastatic melanoma or breast cancer (Dummer et al., 2000). Recently the anticancer effects of recombinant adenovirus p53 (rAd-p53) was assessed on human malignant melanoma. The purpose of this research was to investigate the cellular biological effects of rAd-p53 on tumor cell proliferation and apoptosis, as well as the molecular pathways involved. To further examination, nude mice were used to produce a tumor model and the inhibitory effect of rAd-p53 on the progression of tumors was assessed. Treatment with rAd-p53 exhibited significant inhibitory effects on solid tumor development and metastasis with no side effects and demonstrating the prom therapeutic potential (Shi et al., 2019).

In addition to p53, MDA-7/IL-24, which is a tumor suppressor gene have used for gene therapies in melanoma. Without affecting normal cells or tissues, it exhibits broad-spectrum anticancer action in vitro, in vivo in preclinical studies, and in a phase I/II clinical trial in patients with advanced malignancies. Tumor-specific death by a combination of apoptosis and toxic autophagy, powerful "bystander" anticancer action, immunomodulation, cell proliferation inhibition, and angiogenesis suppression are among the many roles of mda-7/IL-24 in cancer therapy. A recent study shows that mda-7/IL-24 modulates miRNA expression directly in cancer cells, highlighting the importance of the mda-7/IL-24–miR-221–beclin-1 loop in cancer cell death. Results from this study show that after adenoviral-mediated administration of mda-7/IL-24, the level of miR-221 drops significantly in a panel of tumor cell lines. Downregulation of miR-221 is linked to mda-7/IL-24-induced cell death which can be prevented by the overexpression of miR-221. MDA-7/IL-24 production and release in cancer cells, but not in normal cells, lowers cell proliferation and promotes death after treatment with purified recombinant cytokine or infection with Ad.mda-7. Furthermore, through "bystander" anticancer actions, released MDA-7/IL-24 causes death in surrounding cells as well as distant tumor cells (Su et al., 2005). Furthermore, through an autocrine/paracrine loop, MDA-7/IL-24 protein stimulates the production of endogenous MDA-7/IL-24 (Sauane et al., 2008)

Low expression levels of MDA-7/IL-24 in melanoma cells has encouraged researchers to utilized cancer treatment strategies based on this gene and different studies have proven the effectiveness of the MDA-7/IL-24 gene therapy in treating melanoma (Pradhan et al., 2019). MDA-7/IL-24 can induce apoptosis specifically in tumor cells via a reduction of anti-apoptotic proteins such as Bcl-2 and induction pro-apoptotic proteins such as Bax (Menezes et al., 2014). In the case of melanoma, MDA-7 gene delivery by a cancer terminator virus (CTV) to melanoma mice, caused a reduction of tumor-related death in melanoma mice (Sarkar et al., 2008). On the other hand, the safety and strong apoptosis effect of an adenovirus expressing MDA-7/IL-24 gene has been documented in patients with melanoma (Menezes et al., 2014).

4.5. Gene editing tools for therapy

Several gene editing tools have been extended in recent years, including transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), meganucleases (MNs), and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). All of them identify a specific DNA sequence on the genome and their second nuclease creates a double-stranded break in the target sequence. The break repair could introduce insertion/deletion mutations (indels) via the nonhomologous end-joining pathway. Such indels can permanently alter the target gene product if the nuclease object site exists in the coding sequence. Although all technologies differ in the mechanism used to direct the enzyme to the target region, all technology platforms have similar challenges for therapeutic use to achieve the clinical scale cleavage efficiency required for biological effect on the selected human primary tissue/cell while keeping enough target specificity (Beane et al., 2015). CRISPR-Cas is used to delete or insert nucleotides into particular parts of the DNA. This tool is designed easier than other site-specific nucleases. Among gene editing methods, the CRISPR technology has revolutionized gene editing research. This technology allows for the exact change of particular regions in DNA and inspires new techniques for researching and treating inherited disorders by using RNA-guided CRISPR-associated nucleases that are programmable. Most of the research related to gene editing for treating melanoma is based on the CRISPR system. Consequently, here we look at how this tool has aided crucial advancements in melanoma research, argue the importance of this tool in genome editing, and highlight research on CRISPR technologies in treating melanoma. We also look at some of the present CRISPR technologies' limitations and the obstacles these constraints offer for the general use of CRISPR-based therapies (Baker and Hayden, 2020).

An investigation in immunotherapy for diseases like non-small-cell lung cancer and melanoma was one of the first CRISPR-Cas clinical studies in humans (Xu et al., 2020). This study has focused on using gene editing to deactivate inhibitors of key immune checkpoints like two proteins that usually prevent the effect of anti-tumor cytotoxic on endogenous and exogenous T cells, including cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and programmed cell death protein-1 (PD-1) (Buchbinder and Desai, 2016). The first human study was planned to check the usage of CRISPR-Cas9 in melanoma based on the established success of prior immunotherapies, including PD1 (Topalian et al., 2012) inhibitors and T cells transduced with the NYESO1 T cell

receptor (TCR) (Robbins et al., 2011). Investigators hope to promote the therapeutic benefits of current techniques by employing CRISPR-Cas9 to knock out PD-1 gene in autologous NY-ESO-1 TCR-transduced T cells. Autologous T cells are obtained from the patient and transduced with a lenti virus (LV) vector expressing the NY-ESO-1 TCR, priming them to detect melanoma cells that express highly immunogenic NY-ESO-1 antigen (Baylis and McLeod, 2017). Then, they are electroporated with a CRISPR Cas9 vector that can target both PD-1 and the endogenous TCR subunits and disrupt them (Xu et al., 2020).

With the advent of this technique, several CRISPR screens have been performed at the genomic or subgenomic level, nominating new genes and signaling pathways that may make melanoma cells more vulnerable to immune attack (Manguso et al., 2017). In order to recognize new therapeutic targets in melanoma malignancy with altered IFNg signaling, some researchers performed a CRISPR screen using melanoma cell lines lacking the IFNg receptor (IFNGR1) (Vredevoogd et al., 2019). Such screens are worthwhile for screening accessible targets and designating drug candidates for further findings. It is crucial to attend that most of these CRISPR tests to date only assess the effect of gene knockout on cancer cells, which cannot readily explain the result of pharmacological targeting of a specific gene product on different kinds of cells like tumor cells, immune cells and stromal cells existing at the same time (Jenkins and Fisher, 2021).

Various targets have been recognized and demonstrated to be effective in inhibiting melanoma. The aims of these investigations done by CRISPR/Cas9 technology are to find out these genes as novel targets for drug development and to find decent mechanisms of tumorigenesis. The use of CRISPR/Cas9 in studying oncogenic affairs happening during tumorigenesis has revolutionized the investigation in biology of cancer. Significantly, models of CRISPR/Cas9 can support the early steps of drug discovery by contributing to a novel and adequate collection of cancer targets (Behan et al., 2019).

BRAF and NRAS have been identified as the most frequently mutated genes in cutaneous melanoma patients based on genome and exome sequencing studies. Because BRAF and NRAS provoke the MAPK signaling, leading to highly selective kinase inhibitors development that target this signaling. Therefore, understanding the pathways causing to such resistance is crucial (Li et al., 2020; Nagler et al., 2020).

In melanoma, the most common changes among the RAS isoforms are NRAS mutations. Poor prognosis and a lower survival rate are typical in patients with NRAS-mutant melanoma. Recognizing genetic modifiers complicated in resistance to MEK targeted therapy may aid in new therapeutic approaches development and therapeutic response and survival improvement. In a 2019 study, the screen of whole-genome CRISPR-Cas9 knockout recognized the target Kelch domain-containing F-Box protein 42 (FBXO42) as an element in NRAS-mutant melanoma-acquired resistance to the MEK1/2 inhibitor trametinib. The data indicate that FBXO42 plays a crucial role in resistance to the MEK1/2 inhibitor trametinib via the TAK1 signaling pathway in resistance acquired through NRAS-mutated melanoma. These results have beneficial impacts on clinical indications (Nagler et al., 2020).

To recognize remedial target candidates for BRAF inhibitor-resistant melanoma, Li *et al.* performed CRISPR screens to systematically characterize resistance to BRAFi PLX4720 in melanoma. They identified critical regulators of CDK6, such as the ETS family transcription factor ETV5 and JUN family transcription factors, enabling resistance to BRAF inhibitors in melanoma cells. Their results demonstrate genes that contribute to resistance to a selective BRAF inhibitor PLX4720 and provide worthwhile insights into gene regulation in BRAF inhibitor-resistant melanoma cells (Li et al., 2020).

A study done by Giuseppe Ercolano *et al.* used the CRISPR/Cas9 tool for exploring the prostaglandin-endoperoxide synthase 2 (PTGS2) role in the development and progression of melanoma (Ercolano et al., 2019). PTGS2 as the main prostanoid, is mainly expressed in malignant melanomas and directly affect tumor and tumor-stromal cells to promote tumor formation, progression, and metastasis (Nakanishi and Rosenberg, 2013). Collectively, those findings recommend that PTGS2 as a therapeutic target could restrict the immunotherapy resistance and enhance the effectiveness of checkpoint inhibitors in treatment for melanoma. Their study aimed to examine the PTGS2 deletion effects on melanoma expansion and progression. They performed PTGS2 knockdown using CRISPR/Cas9 tool in B16F10 murine cells. Their results demonstrate that reduced expression of PTGS2 causes to inhibition of cell proliferation, migration, and invasiveness. It is noteworthy that subcutaneous tumors as well as metastasis are dramatically reduced in PTGS2 knockdown melanoma models (Ercolano et al., 2019).

In some cases, the CRISPR-Cas system causes activation of silent genes. For instance, PTEN expression is lost in so many cancers. Phosphatase and tensin homolog (PTEN) is a crucial and multifunctional tumor suppressor gene that arrests many cellular processes, including cell cycle progression, survival, and migration (Di Cristofano and Pandolfi, 2000). Loss of PTEN, which happens via several mechanisms, such as mutation induction hands out the development and drug resistance of many cancers like melanoma.

In a 2019 study, researchers stimulated expression of PTEN by the CRISPR technology, specifically dead Cas9 attached to the transactivator VP64-p65-Rta. Using sgRNAs, the dCas9-VPR was directed to the PTEN proximal promoter in cancer cells that revealed low expression of PTEN. This system enhanced expression of PTEN without transcriptional regulation at predicted off-target sgRNA binding sites. The mTOR, AKT, and MAPK as downstream oncogenic signaling pathways are significantly suppressed following PTEN activation. In addition, following treatment with PI3K/mTOR inhibitors, B-Raf inhibitors and a combination of them, migration and colony formation were decreased in the melanoma cells with BRAF V600E mutation that transduced with dCas9-VPR. Moreover, activation of PTEN using CRISPR-Cas9 technology may suggest an alternative therapeutic method for highly aggressive cancers resistant to present treatments (Moses et al., 2019).

Another recent work aimed to develop COX 2 knockout human A2058 cells using CRISPR/Cas9 and then, evaluate the function of the enzyme in the tumor expansion and progression. A pilot protein array study in the knockout cells confirmed that the COX2 enzyme particularly changed downstream signaling pathways and consequently cellular and molecular mechanisms of cancer (Haase-Kohn et al., 2022).

One of the most frequently used stem cells marker in different malignances is CD133. The different investigations demonstrated that CD133(+) melanoma-initiating cells are related to inadequate chemotherapy in patients (C.M. Simbulan-Rosenthal et al., 2019; C.M. Simbulan-Rosenthal et al., 2019). A recent study evaluated the anti-apoptotic activity mechanisms of CD133 in patient-derived BAKP cells after assessing CRISPRCas9-CD133 knockout CD133 expression. Consequently, knockdown of CD133 via siRNA or CRISPRCas9 (BAKRT3) in BAKR cells reduced the level of matrix metalloproteinase MMP2/MMP9 and invasion. In comparison, CD133 knockdown by siRNA or CRISPRCas9 on BAKP cells weakened invasion and decreased MMP2/MMP9 levels, doxycycline-induced expression of CD133 (C.M. Simbulan-Rosenthal et al., 2019). In another investigation which was accomplished in 2022, researchers used the CRISPR-cas9 approach to examine the potential molecular pathways through which CD133 is involved in cell survival elevation and MEKi-trametinib resistance. Melanoma initiating (MIC) cells that overexpress CD133 presented cell viability elevation and apoptosis reduction in response to trametinib. CRISPR-Cas9 inactivation of CD133 reversed this phenomenon in several melanoma cell lines and trametinib sensitized cells. On the other hand, Dox-inducible expression of CD133 reduced trametinib-induced apoptosis and enhanced anti-apoptotic pAKT and BCL-2 family protein levels (Simbulan-Rosenthal et al., 2022). Therefore, CD133 may have a crucial role in invasion and metastasis leading to tumor progression and it seems to be an attractive therapeutic target for melanoma.

Most genetic changes driving melanoma development and resistance to targeted therapies have been discovered. However, little information is discovered about the non-genetic mechanisms driving this cancer

despite their increasingly recognized contribution.

In contrast, little is known about the non-genetic mechanisms driving these processes, despite their increasing involvement.

In outstanding research, researchers used a whole-genome approach, and they looked for signaling pathways that would trigger sustained transcriptional reprogramming of cells in drug-resistant cells. Based on CRISPR screening, data mining, and in vivo experiments, they recognized and verified SLC9A5, BIRC3, and SMAD3 genes to boost both tumor growth and BRAFi-resistance. Their work increases their understanding of melanoma cell biology and highlights new drug vulnerabilities that can be exploited to develop long-lasting therapies against melanoma. These investigators recognize integrated AhR-SMAD3 signaling as a critical driver of growth and relapse in melanoma, indicating a novel therapeutic vulnerability in this cancer (Gautron et al., 2021).

CRISPR-dCas9 has introduced a powerful technology for more targeted editing of DNA methylation. Changes in DNA methylation can modify crucial gene expression, leading to expansion of cancer and metastasis (Chatterjee et al., 2018; Jones, 2012). Jim Smith *et al.* described an effective procedure for targeted editing of DNA methylation in melanoma cells. They studied dCas9-SunTag-based toolkit application to edit the locus of DNA methylation. Furthermore, they represented that methylation and demethylation of the EBF3 promoter, a key player of metastasis, is very effective (Smith et al., 2021).

Several challenges remain to the general application of CRISPR-based therapy. The studies broadly indicate the capability of CRISPR-Cas methods for the treatment of human disease both via in vitro and *ex vivo* modification of primary cells. In vivo strategies would be ideal, but few investigations have been done in this way. However, in vivo dermatology studies where the CRISPR-therapeutics were locally delivered to mouse skin cells have not shown high efficacy or success in long-term follow-up (Xu et al., 2020; Luan et al., 2018; Wu et al., 2017). For in vivo gene editing using CRISPR-Cas9, significant challenges exist yet to be clinically translatable in dermatology and other fields.

The sgRNA must guide the nuclease to a specific location and be optimized with minimal off-target consequences. On the other hand, they should be delivered efficiently to specific human cells and have minimal antigenic characteristics to be accepted by the human immune system. (Cornu et al., 2017). Today, new CRISPR-Cas and gene delivery systems have tried to overcome these problems (Ran et al., 2013; Kleinstiver et al., 2016; Doench et al., 2016). Table 2 provides some recent studies which are investigated gene delivery for melanoma that are under clinical trials.

5. Gene delivery systems for therapy

The most important and challenging issue in gene therapy is efficient gene delivery. The therapeutic gene not only must evade the reticuloendothelial system (RES) when it is introduced into systemic circulation, but also must circumvent numerous barriers before reaching the cytoplasm or nucleus of its target cells (Harris and Elmer, 2021). In fact, the process of delivering a gene typically involves several steps, including DNA/RNA condensation, introduction into the bloodstream, and targeted cell delivery. These steps are followed by cellular uptake, endosomal release, nuclear transport, and the unpacking of the carrier/gene polyplexes, as well as translation in eukaryotic cells (Fig. 2) (Zhang et al., 2012). Each of these processes poses unique obstacles for polyplex delivery, as the physical, chemical, and biological properties of the carrier must support all of these extracellular and intracellular steps (Jinturkar and Misra, 2011). Therefore, it is essential to carefully evaluate the major mechanisms and challenges of each of these phases in order to develop more therapeutically effective gene delivery methods with fewer side effects.

To date, three types of gene delivery systems have been investigated, including modified naked genes (e.g. siRNA, plasmids, and so on), viral vectors, and nonviral vectors. Modified naked genes, compared to unmodified genes, have generally enhanced nuclease stability and gene silencing efficiency, as well as minimized immune responses and off-target effects. However, the transfection efficiency is not sufficient (Yahya and Alqadhi, 2021). Viral vectors, on the other hand, have high transfection efficiency, but their residual viral elements can stimulate mutation and immune responses (Duncan, 2022). Nonviral vectors, as the newest strategy in gene delivery, are fabricated with biocompatible materials using rational and safe approaches. Therefore, they can faithfully deliver gene cargo into targeted cells (Li et al., 2019). Despite the fact that their transfection effectiveness is not as great as viral vectors, nonviral vectors are becoming more popular due to their safety, scaled-up production, and low cost. In light of this, developing an appropriate delivery strategy that transfects genes to the targeted sites following local or systemic distribution is a major issue and represents the most important barrier between gene technology and therapeutic application (Amreddy et al., 2018). In the following section, we present available and faithful strategies for effective gene delivery in melanoma therapy.

5.1. Nano-Based gene delivery

Nanomedicine is the study and design of materials at the nanoscale, and it has been studied extensively in recent decades in order to develop

Table 2
Recent investigates for gene delivery in melanoma which are under clinical trails.

Therapeutic agent	Cell	Description	Status	Clinical trail
Allovectin-7®	–	The goal of this clinical research is to ascertain whether the experimental gene-based immunotherapy Allovectin-7® can reduce melanoma tumor size. The experiment will also look at whether this therapy can delay the onset of the disease.	Phase II	NCT00044356
F5 TCR	Dendritic cells	The apheresis product will create gene-modified MART-1 TCR CTLs and dendritic cells, which will be tested to ensure they express the proper TCR.	Phase II	NCT00910650
CTL	Artificial antigen presenting cell (aAPC)	The study is investigating the feasibility and side effects of intravenous infusions of laboratory-produced CTL, which is produced through leukapheresis and combined with extra genes.	Phase I	NCT00512889
GVAX	Clear Cell Sarcomaprocedia	Procedia#apol14p	Phase I	NCT00258687
HBI 0201 /ESO TCRT		HBI 0201-ESO TCRT (anti-NY-ESO-1 TCR-Gene Engineered Lymphocytes) Given by Infusion to Patients with NY-ESO-1 -Expressing Metastatic Cancers: A Phase I/II Dose Escalation, Safety and Efficacy Study.	Phase I/ II	NCT05296564
HX008/OH_2	Granulocyte macrophage	Herpes simplex virus type 2 strain HG52 underwent genetic alterations to become OH_2, an oncolytic virus that can only replicate in tumors. A stronger antitumor immune response may be induced by the transfer of the gene encoding human granulocyte macrophage colony-stimulating factor (GM-CSF).	Phase I/ II	NCT04616443
RNA/Lipo-MERIT	T cells	Tyrosinase, Melanoma-Associated Antigen A3 (MAGE-A3), New York-ESO 1 (NY-ESO-1), and Trans-membrane phosphatase with Tensin Homology (TPTE) are the four selected malignant melanoma-associated antigens that the Lipo-MERIT vaccine is designed to target.	Phase I	NCT02410733

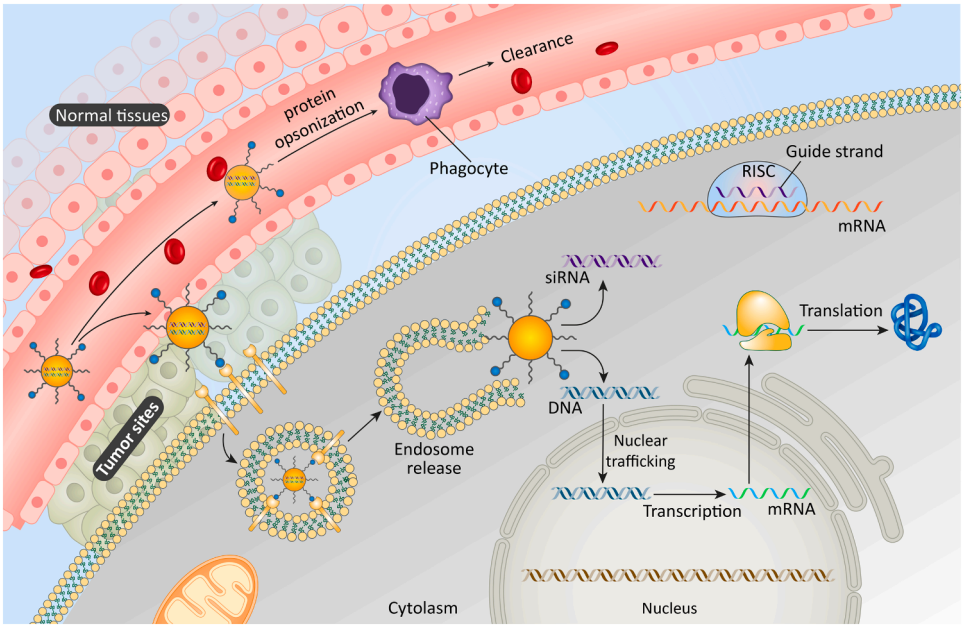

Fig. 2. Illustration of the process and major obstacles in gene delivery for melanoma, including production of the nucleic acid polyplex, systemic circulation, accumulation of the target tissue, intracellular transit, and nucleus unpacking.

efficient delivery methods for diagnostics and therapies in a variety of disorders. High intratumoral pressure caused by stromal cells, poor perfusion, drug/gene efflux, and intracellular trapping have all been attributed to chemotherapeutic resistance in melanoma, resulting in ineffective drug/gene delivery. Sentinel lymph node imaging, chemotherapy, and RNA interference are only a few of the applications of nanomedicine in the treatment of melanoma. Each of these applications has the potential to develop efficient and individualized diagnostic and therapeutic procedures. In the following section, the most investigated nanoparticles as effective nanovectors in melanoma therapy have been overviewed.

5.1.1. Polymeric-Based nanovectors

Polymer-based gene delivery approaches are a set of non-viral vectors with a wide range of applications. Cationic polymers can easily form spherical complexes (= polyplexes; 50–200 nm in diameter) with negatively charged genetic cargo thanks to electrostatic interactions (Blakney et al., 2020). The most often used polymers for skin applications are typically polyethyleneimine (PEI) and poly- (-amino ester). A number of in vitro and in vivo studies have shown that PEI-based nanovectors may be able to enhance and treat skin disorders because of their high transfection effectiveness and excellent endosomal escape. The severe toxicity of PEI, on the other hand, is widely reported (Malloggi et al., 2015). Given that the molecular weight (MW) of cationic polymers is directly connected to their transfection efficacy and cytotoxicity, it is noteworthy that a high MW PEI correlates with high transfection efficacy and toxicity. In contrast, branched PEI complexes transfer genetic material 15 times more efficiently than linear PEI at the same MW (B.F. Craciun et al., 2019) Similar to PEI alone, PLGA [poly (lactic-co-glycolic acid)] and PEI together successfully encapsulated and transfected siRNA into dendritic cells, restoring cell maturation and functionality and significantly increasing allogeneic T-cell proliferation in vitro (Alshamsan et al., 2010). In a study, a new vaccine platform for tumor immunotherapy was developed using polyethylene glycol polymer (PEGylated) tumor cell membrane vesicles, which was validated in a mouse tumor model. PEGylated NPs were formed by using the endogenous cell membrane obtained from cancer cells (Ochyl et al., 2018). PEG-NPs exhibited good serum stability in vitro and efficient drainage through local lymph nodes when administered subcutaneously in vivo. When tested on tumor-bearing mice, treatment with PEG-NPs synthesized by mouse melanoma cells resulted in high-efficiency antigen-specific cytotoxic CD_8^+ T lymphocyte responses.

Another reliable method for effective gene delivery is the use of "smart" polymers that adapt to changes in the biological environment. One study, for instance, evaluated the effect of fluorination and the bioreducibility of cationic hyperbranched polymers (amido amines) (Chen et al., 2017; Chen et al., 2018). After intravenous and tumoral injections in mice, it was discovered that reducing fluorinated polymers successfully conjugated with siRNA, leading to stronger gene knockdown than nonreducing polyplexes. Another study team developed pH- and redox-sensitive nanoparticles (NPs) employing a galactose-functionalized n-butylamine-poly(L-lysine)-bpoly(L-cysteine) polypeptide core coated with PEG copolymers for the targeted delivery of miR155 to tumor-associated macrophages (Liu et al., 2017). These polyplexes successfully transfected tumor-associated macrophages and raised miR155 expression by 100–400% in melanoma xenografted mouse models after intratumoral injection and in vitro experiments. Notably, despite the fact that PEGlation was effectively introduced to extend circulation, the "PEG dilemma" may also hinder cellular uptake and gene release (Hatakeyama et al., 2011). Considering this designing a rational nanovectors to circumvent this limitation could be a promising approach. More recently, a study team presented a unique polymeric core-shell made of negatively charged multifunctional RRPH (RGD-R8-PEG-HA) shell and fluorinated polymers (PFs) core binding with plasmid DNA. Particularly efficient PFs included self-nucleus targeting, endosomal escape, and plasmid DNA condensing (Fig. 3A) (L. Li et al., 2016). RRPH in this device provides depth penetration, multi-stage, and multiple tumor-targeting capabilities. Hence, conforming to the in vitro and in vivo findings, the therapeutic efficacy of RRPHC ternary complexes loaded with therapeutic genes (mTRAIL) was impressive, and melanoma was greatly suppressed. As a result, the RRPHC ternary complexes could be a viable delivery strategy for melanoma-specific gene delivery (L. Li et al., 2016).

Biobased polymer nanoparticles, such as a poly(thioether-ester)-PTe nanoparticles, display excellent biocompatibility, biodegradability and antioxidant proprieties and have been candidates for biomedical applications (Cardoso et al., 2018). Recently, the bioformulation of PTEe nanoparticles containing full-spectrum Cannabis extract have been

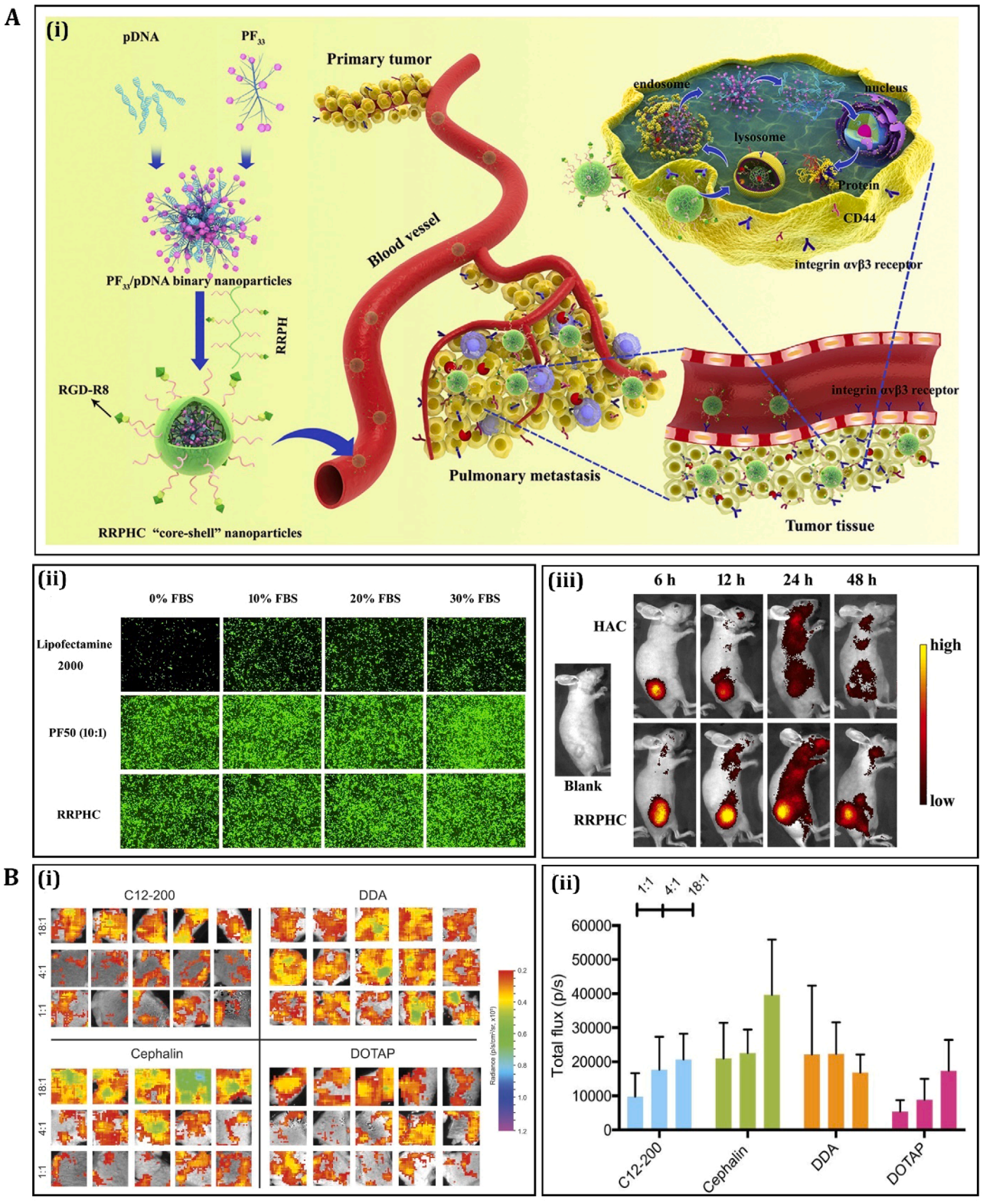

Fig. 3. Recent works on the polymeric-based and lipid-based nanovectors for gene delivery in melanoma: (A, i) Schematic illustration of the RRPHC ternary complexes **(ii)** In B16F10 cells, the transfection efficiency of PF33/pGFP, RRPHC/pGFP, and Lipofectamine 2000/pGFP, **(iii)** Intravenous injection of HAC/pDNA (HAC), RRPHC/pDNA, in vivo fluorescence imaging and *ex vivo* fluorescence imaging of A375 tumor-bearing nude mice (RRPHC). Reproduced with permission from ref (L. Li et al., 2016) Copyright 2016 Elsevier. **(B)** Human skin explants were injected intradermally with LNP formulations with varied lipid identities and lipid nanoparticle doses **(i)** *Ex vivo* imaging of explants after 11 days, and **(ii)** The luciferase image was quantified. Reproduced with permission from ref (Blakney et al., 2019) Copyright 2019 American Chemical Society.

studies for their anticancer proprieties. Interestingly, these nanostructures could release cannabis molecules in cellular models and display strong antitumor activity in BF16F10 melanoma cells by regulating signaling pathways, mitochondria biology and autophagy (Freire et al., 2023).

Poly (beta-amino ester)s (PBAEs) are another type of cationic polymer that can deliver genes into the skin with a high transfection efficiency comparable to viral vectors. More than 2000 diverse linear PBAEs have been developed so far, with some showing superior results to PEI and viral vectors (Green et al., 2008; Green et al., 2007). As described before, the effectiveness of transfection is considerably increased by branching the cationic polymers (D. Zhou et al., 2016). For instance, ex vivo investigation shown that highly branched PBAE-based NPs effectively supplied keratinocytes isolated from EB patients with minicircle DNA encoding for COL7A1 (Zeng et al., 2019). Similarly, in EB models, topically administered PBAE NPs effectively restored collagen VII production while causing less cytotoxicity than PEI (D. Zhou et al., 2016). These promising outcomes led to the approval of the first topical gene therapy product candidate based on highly branched poly (amino esters) (HPBAEs) in 2018. In general, polymeric NPs are being used more frequently to deliver genes to the skin. However, it should be noted that most research is still in the preclinical stage, which confuses the actual translational value (Hainzl et al., 2017; Wang et al., 2020). Despite polymeric NPs missing the natural infective features of viruses, we have made progress in the development of stable, flexible, and highly effective gene delivery techniques by combining our expertise in cell biology and bioengineering of synthetic biomaterials (Lostalé-Seijo and Montenegro, 2018). Ineffective transfection efficacies relative to viral vectors and effective endosomal escape have hindered the clinical translation of polymeric NPs. Notably, a clinical trial (NCT02730766) is currently underway to investigate the use of polymer-based nanoparticles for gene therapy in melanoma. The trial is evaluating the safety and efficacy of the polymer-based nanoparticles loaded with the plasmid DNA encoding for the melanoma antigen NY-ESO-1.

5.1.2. Lipid-Based nanovectors

Non-viral gene delivery techniques based on lipid-based nanoparticles (LNPs) are now the most faithful nanovectors. LNPs are now a widely used platform technology in medicine for delivering genetic material with different sizes (Fink et al., 2006).

Only a few studies have so far looked at the use of LNPs to treat skin conditions. As an example, cationic lipids [such 1,2-dioleoyl-3-trimethylammonium propane (DOTAP)] have historically been used in conjunction with certain lipids (like phospholipids or cholesterol) to complex, store, and carry nucleic acids (Buck et al., 2019). These lipoplexes, as well as lipofectamine, are effective agents for delivering therapeutic genes to cells. Despite their promise, these systems have limited therapeutic applicability due to carrier-related toxicity and immunological activation caused by the permanent cationic charge. Furthermore, several cationic lipids, such as DOTAP, have been demonstrated to hinder effective dermal gene transfer (Blakney et al., 2019). The discovery of ionizable cationic lipids like DLin-MC3-DM was a major step forward in LNP technology (Semple et al., 2010). The acid dissociation constant (pKa) of this class of lipids is approximately 6.5. This maintains the lipid positively charged at acidic pH and neutral under physiological conditions, enabling efficient nucleic acid trapping (Jayaraman et al., 2012). The ionizable lipid is more easily protonated by the low endosomal pH, which causes endosomal instability through interactions with negatively charged endosomal lipids and ultimately results in cytoplasmic release of the genetic cargo (Kulkarni et al., 2019). Consequently, the ionizable lipid serves three purposes: effective interaction and trapping with therapeutic nucleic acid, particle toxicity reduction, and endosomal escape facilitation (Jayaraman et al., 2012). The delivery of gene-editing complexes using LNP technology has a lot of potential as a treatment for skin issues. A recent study found that the kind of complexing lipid had a considerable impact on the delivery of self-amplifying messenger RNA to human skin explants (Fig. 3B) (Blakney et al., 2019). Furthermore, a research group have shown that quaternary ammonium-based lipoplexes have the potential to induce a specific immune response, making them suitable for use as a cancer immunotherapy vaccine. The mRNA-lipoplexes consist of DOTMA/-DOPE or DOTAP/DOPE lipids, which protect the mRNA encoding the antigen from extracellular ribonucleases. Upon systemic administration, lipoplex (DOTAP/DOPE) accumulates efficiently in the spleen and delivers mRNA to dendritic cells. A phase I dose-escalation trial is underway, where three melanoma patients were initially treated with a low dose of RNA-lipoplexes and produced IFNα and strong antigen-specific T cell responses. The next phase produced vaccines with RNA-lipoplexes encoding melanoma-associated malignant antigens, such as New York-ESO 1 (NY-ESO-1), tyrosinase, melanoma-associated antigen A3 (MAGE-A3), and transmembrane phosphatase with tensin homology (TPTE) (NCT02410733). Another clinical trial (NCT00689065) evaluated the use of lipid-based nanoparticles loaded with siRNA targeting the protein polo-like kinase 1 (PLK1) in patients with advanced solid tumors including melanoma. The results of the trial showed that the lipid-based nanoparticles were safe and well-tolerated, and led to a decrease in PLK1 expression in some patients.

5.1.3. Carbon-Based nanovectors

Single-walled carbon nanotubes, fullerene, graphene, and nanodiamonds are examples of carbon nanoparticles that have high photostability (their fluorescence does not change even after continuous photobleaching), low toxicity, and good biocompatibility, making them promising agents for therapeutic and diagnostic uses (Gholami et al., 2020). In addition, these nanoparticles could be eliminated from the body efficiently and quickly. Consequently, carbon nanoparticles have a lot of potential in biomedical research including faithful carriers for efficient drug/gene delivery. Indeed, carbon-based nanoparticles have a high capacity for gene loading because of their unique structure and chemistry (Luo et al., 2018). The surfaces of carbon-based nanoparticles and its derivatives have been modified with various polymers or ligands to improve biocompatibility, biostability, cellular uptake, and gene loading capacity. For instance, a research group functionalized carbon nanoparticles with chitosan polymer for encapsulating thiolated siRNA targeting Plk1 gene (siPlk1) to form C-siPlk1 (Zhang et al., 2016). The C-siPlk1 showed great performance in melanoma cells (A375), extremely effective down-regulation of the Plk1 gene, and significant tumor suppression effects, according to in vitro and in vivo studies. The C-siPlk1 has a variety of functionalities overall thanks to the therapeutic siRNA shell and fluorescent carbon nanoparticles core, including simple imaging and tracing. In another study, a new type of NP, called NP-doped carbon dots (CDs), which were composed of carbon dots doped with magnesium oxide (MgO) and PEG. This study focuses on the development of a multifunctional nanoparticle (NP) system that regulates apoptosis and autophagy in B16F10 melanoma cancer cells and can be used for in vitro imaging applications (Bajpai et al., 2020). The results showed that the NP-CDs had good biocompatibility and low cytotoxicity in normal cells but demonstrated a significant inhibitory effect on B16F10 melanoma cancer cells. They could induce apoptosis and autophagy in these cells and cause a decrease in cell viability. The authors also demonstrated that the NP-CDs could be used for in vitro imaging applications due to their strong fluorescence emission under UV light (Bajpai et al., 2020).

5.1.4. Silica-Based nanovectors

Mesoporous silica nanoparticles can be modified with diverse substances for a variety of clinical uses, including effective drug/gene delivery (Sharifi et al., 2021). These nanoparticles have a greater surface-to-volume ratio, increasing functionalization while retaining porosity, which allows a significant amount of carrier to transport therapeutic drugs without weakening the silica framework. The current improvement in nanomedicines has assisted in the large-scale

development of mesoporous silica nanoparticles. These nanomaterials have been found to be useful in the treatment of skin cancer in various investigations (Zhou et al., 2018).

One of the most common nanomaterials, amorphous silica nanoparticles, can penetrate the skin and have the desired impact (Hooshmand et al., 2021). It was indicated that amorphous silica nanoparticles can produce immunomodulating effects in mice with atopic dermatitis that can penetrate the skin A research group encapsulated siRNA into mesoporous silica nanoparticles to treat squamous cell carcinoma as a type of skin problem. The results revealed a two-fold suppression of TGFβR-1 in a mouse xenograft model (Lio et al., 2019). According to the literature, dendritic and epidermal cells were often linked to the topical administration of silica-based carriers. For instance, study of the skin layers is crucial to determining the penetration of nanoparticles in patients with eczema and atopic dermatitis (Rancan et al., 2012).

5.1.5. Gold-Based nanovectors

Gold nanoparticles (AuNPs) are another widely used and applied platform for treating, diagnosing, and monitoring skin disorders, such as melanoma. Surface-modified gold nanoparticles are regarded as efficient gene delivery agents due to their small size, general non-toxicity, simplicity of functionalization, and high surface-to-volume ratio (Niu et al., 2017). Gold nanoparticles with localized surface plasmon resonance enable non-viral siRNA gene delivery to be better considered.

In this regard, AuNPs chemically modified with low molecular branched polyethylenimine (bPEI) have been employed for the efficient delivery in cancer cell lines of therapeutic nucleic acid (gapmers) targeting p53 mutant protein (García-Garrido et al., 2021). In another study, albumin-stabilized gold nanoparticles (ABNs) have been explored as carrier for AZD8055, an mTOR inhibitor for the treatment of uveal melanoma (Latorre et al., 2021). These nanoformulations were able to release the drug upon high concentration of glutathione, as frequently occur inside tumoral cells, and provided excellent cytotoxic effect in uveal melanoma cell lines and tumor growth inhibition in animal models (Latorre et al., 2021).

Up to now, melanoma cells have been used to study the effects of subcutaneous perforation and transfection of plasmid DNA utilizing multifunctional carrier AuNP (Kumar et al., 2019). For instance, AuNPs modified with a cell penetrating peptide and cationic polymer (PEI)

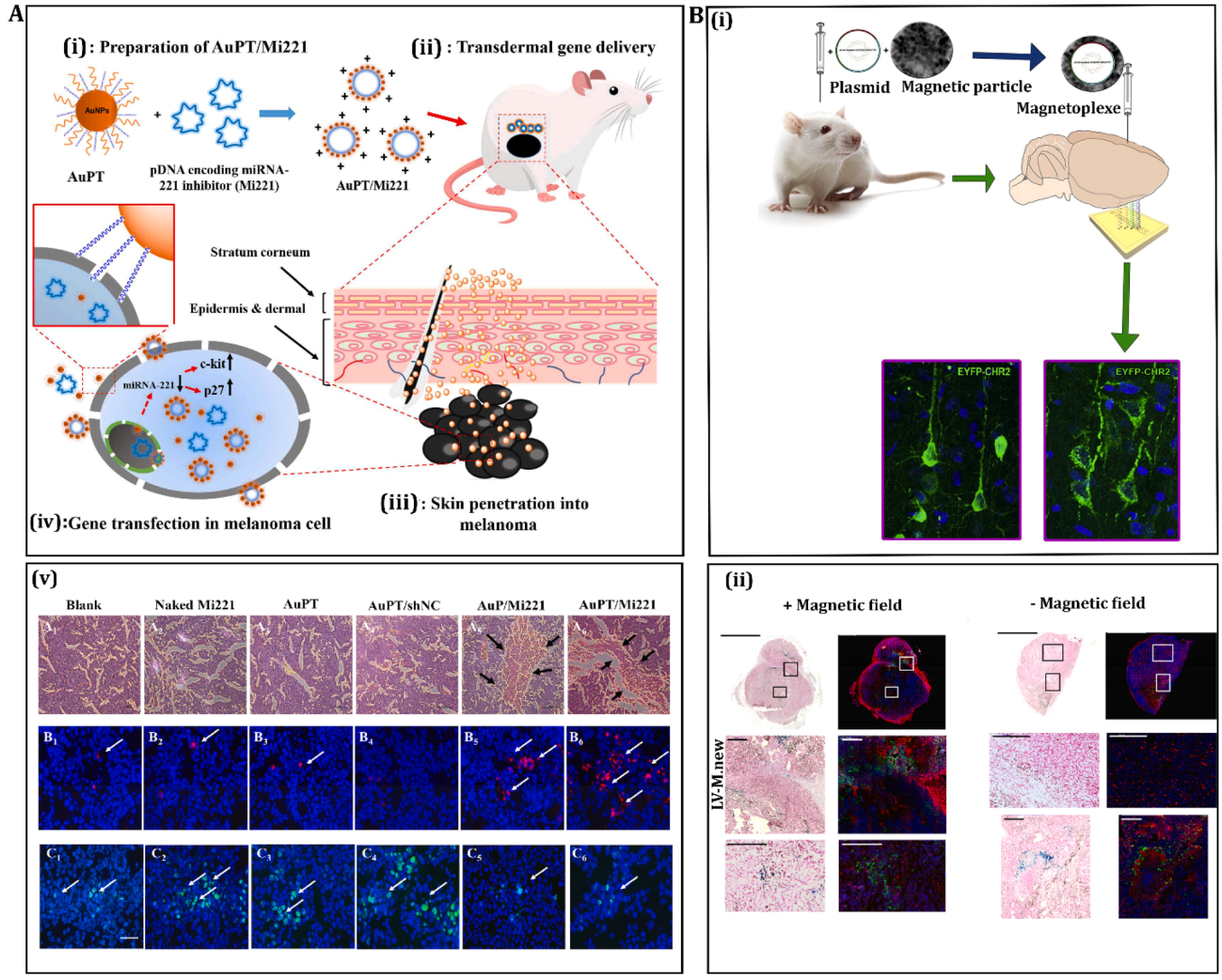

Fig. 4. Recent works on the gold-based-and magnetic-based nanovectors for gene delivery in melanoma: (A) Schematic diagram of the transdermal delivery of plasmid DNAs encoding microRNA-221 inhibitor gene (Mi221) via AuPT nanoparticles for skin cutaneous melanoma treatment. Reproduced with permission from ref (Shahbazi et al., 2016) Copyright 2017 American Chemical Society. (B, i) Local injection of MNP-based gene carriers followed by external magnetic attraction ensures long-term and efficient gene delivery. Reproduced with permission from ref (Borroni et al., 2017) Copyright 2015 Elsevier. (ii) GFP expression in tumors after in situ injection of lentiviral vectors-magnetic nanoparticles (LV-MNPs). Reproduced with permission from ref (Borroni et al., 2017) Copyright 2017 Elsevier.

were evaluated to facilitate the skin penetration of plasmid DNA deeply into the melanoma tissues (Ihle et al., 2015). According to the results, the plasmid DNA was totally able to condense into cationic nanocomplexes and pass through the intact stratum corneum without the need for any extra improvement. Additionally, AuNP strongly promotes the nuclear targeting and intracellular uptake of plasmid DNA in cells, resulting in efficient transfection. The delivery of a plasmid DNA encoding a miRNA-221 inhibitor gene via HIV-1 twin-arginine translocation peptide attached cationic AuNPs was also found to be a feasible candidate for tumor suppression in melanoma. Indeed, miRNA-221 reduces viability and induces apoptosis mediated by the BCL2, KIT, and AKT signaling cascade (Fig. 4A) (Shahbazi et al., 2016). Overall, according to the literature, AuNPs-based nanovectors could circumvent the problems associated with targeted gene delivery to the tumor site located in the skin subcutaneous layer, making them a promising vector in clinical applications.

5.1.6. Magnetic-Based nanovectors

Superparamagnetic iron-oxide NPs (SPION) owing to their increased drug encapsulating capacity, intense magnetic responsiveness, and targeted delivery efficiency, have been studied for drug/gene targeting and other biomedical applications (Gonçalves et al., 2017). According to the literature, skin composition and structure will not allow molecules with a molecular weight greater than 600 Da (>600 Da) to pass through the skin layers. The development of several types of magnetic nanoparticles with a few nanometers has become easier because of recent breakthroughs in nanomedicine (Rao et al., 2015). Many studies have shown that these specific nanomaterials can penetrate the stratum corneum and hair follicles through the epidermis For instance, a study team investigated the interactions of biocompatible cationic amino ultra-small superparamagnetic iron oxide nanoparticles with human cells in various dimension cultures using electron microscopy and biochemical methods (2D and 3D)[252]. According to the findings, human melanoma cells internalized the amino-SPIONs efficiently. The uptake pathway was also found to be clathrin-regulated and localized in the lysosome, which stimulates and decreases the expression of cathepsin D and transferrin receptors in skin fibroblasts being tested for skin melanoma (Cengelli et al., 2010).

It is significant to note that SPIONs have already shown promise for application in cancer gene therapy using magnetofection. Through the process of magnetofection, functionalized SPIONs containing nucleic acids are delivered to the desired cells and help the nucleic acids enter the cells more easily. In preclinical in vitro and in vivo studies, magnetofection has already proven to be an efficient nonviral transfection method. Likewise, it was revealed that small interfering siRNA, plasmids, short hairpin shRNA, and antisense oligonucleotides can all be transfected with it. It should be noted that SPIONs' chemical and physical characteristics are essential for achieving a high cell survival rate and significant magnetofection efficiency with a high cell survival rate. For instance, it was indicated that the semi-solid formulation of magnetic nanoparticles could be a proper candidate for human skin penetration to target different skin diseases (Filon et al., 2015). Isolated stratum corneum or human epidermis were used to test the in vitro penetration of magnetic nanoparticles aggregates. When compared to cold cream, the Cet cream formulation improved penetration and reduced retention, favoring accumulation into the skin membrane. The findings showed that the skin semi-solid formulation may be employed to deliver magnetic nanoparticles without disrupting the skin's permeation pattern. Another research group investigated different parameters on the synthesis of SPIONs and their transfection efficiency (Prosen et al., 2013). They found that altering the manufacturing procedure's parameters had little effect on the effectiveness of magnetofection, but that modifying with poly(acrylic) acid (PAA) and PEI significantly enhanced plasmid DNA-encoded gene expression in melanoma cells. Additionally, they showed that a crucial factor in achieving high gene expression for SPIONs-PAA functionalization is the pH of the PEI water solution (Prosen et al., 2013). This strategy was also used to conjugate magnetic nanoparticles to lentiviral vectors with or without a silica shell targeting gene expression in melanoma tumor cells (Fig. 4B). They indicated that green fluorescent protein expression was sustained for a long time after intravenously infusing lentiviral vectors-magnetic nanoparticles complexes. Besides, green fluorescent positive cells increased in the livers and spleens of intravenous injected mice when a magnetic field was applied to the abdomen (Borroni et al., 2017).

5.2. Physical gene delivery

While non-viral delivery systems have gained immense attention, physical non-viral gene delivery approaches have shown promise for transfecting difficult-to-transfect cells in skin. Physical gene delivery approaches seek to transport nucleic acids (DNA/RNA) directly to the targeted cell while avoiding issues including immunogenicity and endocytic routs. Nonetheless, the physical non-viral gene delivery strategy has its own pros and cons, restricting its application for clinical purposes. In the following, the most applicable physical gene delivery methods in melanoma therapy are reviewed along with their advantages and limitations.

5.2.1. Electroporation

Electroporation is a successful method that has received the greatest attention and is frequently utilized for gene delivery across the skin in vitro and in vivo (Argus et al., 2017). It actually uses high-intensity electrical force to transport therapeutic nucleic acids from extracellular compartments into cells, which creates transient pores in the cell membrane, resulting in the cellular entry of nucleic acids with high transfection efficiencies. This electric power pulse is carried out to the cells among the electrodes to create small membrane pores throughout the membrane in three milliseconds (ms), which might also increase as much as 120 nm in 20 ms (Lesueur et al., 2016). These pores are temporary, resealing within seconds to minutes without causing any major damage to the exposed cells' membranes. By using the local electrophoretic effect, a wide range of therapeutic nucleic acids could be introduced into the exposed cells during this time. According to the literature, electroporation is used to transfect a variety of cell types with high efficiency, stability, and reproducibility (Pereira et al., 2017). For instance, delivery of cDNA and plasmids encoding therapeutic genes using electroporation has been widely evaluated in preclinical melanoma models with both intratumor and alternative tissue. So far, many studies have been conducted with directed gene delivery strategies, including cytokine plasmid DNA delivery in the Cloudman mouse melanoma model, delivery of plasmid DNA encoding dominant negative Stat3, as well as delivery of genes or siRNA to downregulate genes important to cell growth have also been investigated in melanoma therapies (Bolhassani et al., 2014). The results of these studies revealed that direct delivery to the tumor typically generates a direct antitumor effect. In cases where tumors aren't electrically accessible, however, delivery to alternative tissues with systemic expression would be quite beneficial. In this case, systemic transgene expression can also occur after electroporation-mediated plasmid delivery to muscle or skin. Numerous preclinical research studies have been evaluated the intradermal or intramuscular electroporation mediated plasmid delivery for most cancers (Heller and Heller, 2010). Their investigations have indicated that intradermal delivery of plasmids encoding survivin epitopes or intramuscular delivery of plasmids encoding the immune modulators IL-12, interferon, IL-2, and the anti-angiogenic protein vasostatin did not result in regression of palpable melanomas (Greaney et al., 2020). However, tumor growth suppression, enhanced survival, avoidance of metastases, and other anticancer benefits have been demonstrated in most studies. Another promising strategy for melanoma therapy is the combined delivery of nucleic acids using both intratumoral delivery and intramuscular delivery simultaneously. More recently, a research group used intratumoral delivery of plasmids encoding anti-angiogenic

proteins in combination with intramuscular delivery of plasmids encoding tumor antigens, resulting in the induction of regressions in 57% of tumors (Canton et al., 2017).

It is noteworthy to mention that, despite the high efficiency of the electroporation technique, it possesses some drawbacks, including the fact that it can only be applied to suspension cells and that it creates cytotoxicity related to pH change (Shirley et al., 2014). These limitations prevent the widespread application of electroporation in vivo and in clinical evaluation for melanoma therapy. Notably, iontophoresis, which uses low-intensity electrical currents to enhance and direct the cell penetration of charged molecules, can be used to get around these restrictions. For instance, non-invasive iontophoresis was used to effectively deliver STAT3 siRNA as intratumoral injections in melanoma mice models (Labala et al., 2017). Additionally, they used gold nanovectors to co-deliver chemotherapeutic drugs to increase anti-tumor activity and decrease cancer resistance. The findings revealed that in comparison to STAT3 siRNA or imatinib mesylate loaded gold nanovectors, co-delivery of STAT3 siRNA and imatinib mesylate encapsulated in gold-based nanovectors resulted in a significant (p 0.05) reduction in percentage tumor volume, tumor weight, and inhibited STAT3 protein expression (Fig. 5A) (Labala et al., 2017). Hence, the co-delivery strategy based on nanovectors along with electroporation is a promising strategy in melanoma therapy.

5.2.2. Sonoporation

Sonoporation (ultrasound) can also be applied to successfully deliver genes through biological barriers (Escoffre et al., 2013). Sonoporation transiently permeabilizes the cell membrane by generating cavitation bubbles, allowing genetic cargo to enter cells more easily. Typically, low and medium ultrasonic frequencies are employed for intradermal distribution, resulting in high 'cavitational effects" and the creation of vapor-filled bubbles, which eventually collapse and generate pores in the cell membrane (Chowdhury et al., 2020). Microbubbles may be useful for sonoporation in human trials because they are projected to improve gene transfer at a lower mechanical index for patient safety. With a lower pulse frequency and higher acoustic pressure and pulse length, microbubbles are eliminated more effectively (Schwartz et al., 2021). In another work, sonoporation strategy for anti-melanoma DNA vaccination was introduced using antigen presenting cell (APC)- selective gene carrier Mannose- polyethylene glycol (PEG) 2000 lipoplexes (Man-PEG) (Fig. 5B) (Un et al., 2011). Sonoporation of a plasmid DNA encoding the ubiquitylated melanoma-specific antigens gp100 and TRP-2 (pUb-M) with Man-PEG was used to promote immunity. These approaches for sonoporation immunogene delivery highlight the potential for sonotherapy to play a key role in the development of nonviral gene delivery approaches, particularly those that can deliver potent immunostimulatory molecules.

Since cavitation does not occur consistently and cannot be regulated in skin tissue, this approach has a lower transfection effectiveness than electroporation. To address these limitations, the use of complexes with chemicals and diagnostic ultrasound are promising approaches. Indeed, real-time monitoring of the irradiation field is desirable to efficiently transfer therapeutic genes to target tissues (Yang et al., 2021). Overall, various parameters influence the transfection efficiency of sonoporation, including the frequency and intensity of ultrasound waves, ultrasound pressure, and exposure time. An ultrasonic contrast agent is another important parameter, which can decrease the threshold for ultrasound cavitation and hence improve transfection efficiency (B.F. Craciun et al., 2019). However, cavitation within the tissues is rarely precisely controlled. Consequently, increasing cavitation uniformity and cell contrast precision could improve sonoporation transfection efficiency.

5.2.3. Microneedles

The microneedle, a new form of transdermal delivery, has been investigated to increase the transferability of molecules into the skin. In fact, microneedles (50–1500 μm) overcome the stratum corneum by forming microscopic pores, allowing the therapeutic molecules to directly bypass the entire stratum corneum and enter the tumor site (Singh and Kesharwani, 2021). The microneedle technique is superior to previous intradermal administration systems in several ways, including ease of manufacture, handling, and use, in addition to safety and painlessness. There are different types of microneedles, including hollow, solid, dissolved, and coated microneedles, that have been applied for skin cancer treatment, including basal cell carcinoma, melanoma, and squamous cell carcinoma (Zhi et al., 2021). Microneedles have typically been combined with other materials such as biomolecules, nucleic acids, and drugs, regardless of the type of microneedle. Due to their enzyme instabilities, particularly for gene delivery, these materials have been combined with polymers and/or nanomaterials and mediated into the skin using different microneedles. Notably, it was indicated that hollow microneedles seem promising for the topical delivery of different nucleic acids, such as DNA, and siRNA (Chen et al., 2022). As an example, they were able to transfer STAT3 siRNA into melanoma mice models by intradermal administration, which reduced STAT3 messenger RNA expression by 60% and tumor weight and volume by 80% (Pan et al., 2018). To test siRNA's functional stability, a study team coated steel microneedles with the appropriate concentrations of siRNA (Chong et al., 2013). In order to enhance in vivo local gene silencing, it was discovered that the siRNA coated on the steel microneedles could still function well and could be effectively released from the steel microneedles and carried into the skin. These results suggested that a simple, minimally invasive, and patient-friendly method for melanoma gene therapy would include coating steel microneedles with genes (Chong et al., 2013). In another work, steel microneedles modified with cell penetrating peptide octaarginine (R8) nano-complexes containing BRAF siRNA (R8/siBraf) was used for targeted anti-melanoma treatment (Fig. 5C) (Ruan et al., 2018). The results demonstrated that R8/siBraf coated microneedles could substantially improve R8/siBraf transfection and dramatically reduce melanoma growth, inducing tumor cell apoptosis and decreasing their proliferation. It should be noted that the restricted loading capacity and the difficulty with whole-body administration are the main limitations for clinical applications, which should be considered (Chen, 2018).

6. Future perspective

The studies described in this article reassess melanoma pathogenesis and the genetic pathway underlying its formation. Besides, finding the best way to treat this devastating cancer amongst its conventional and advanced therapies known up to now was the main goal of this article, though there are still several questions to be answered. To date, it has been recognized that monotherapy is likely to fail due to the aggressiveness and high variability of melanoma tumors. On the other hand, the data on patients treated with combination therapy are insufficient to reach a definitive decision since the toxicity and resistance issues have not been assessed completely. Today, advanced melanoma therapies such as nanomedicine or gene editing tools and nucleic acid-based therapies as well as investigating the molecular pathways involved in them have created a promising perspective in treatment methods. Hence, the investigation of new aspects of these treatments is required to provide better clinical therapy for melanoma patients and improve their prognoses.

Author contributions

A.R.: Investigation, Writing - review & editing, Y.E.: Conceptualization; writing-orginal draft; Writing - review & editing, N.D.: Investigation; writing-orginal draft, A.D.: Investigation; writing-orginal draft, I. R.: Investigation; writing-orginal draft, S.J.: Investigation; writing-orginal draft, G.V.: Investigation; writing-orginal draft, L.S.: Funding acquisition; Resources; Writing - review & editing, A.Z.:

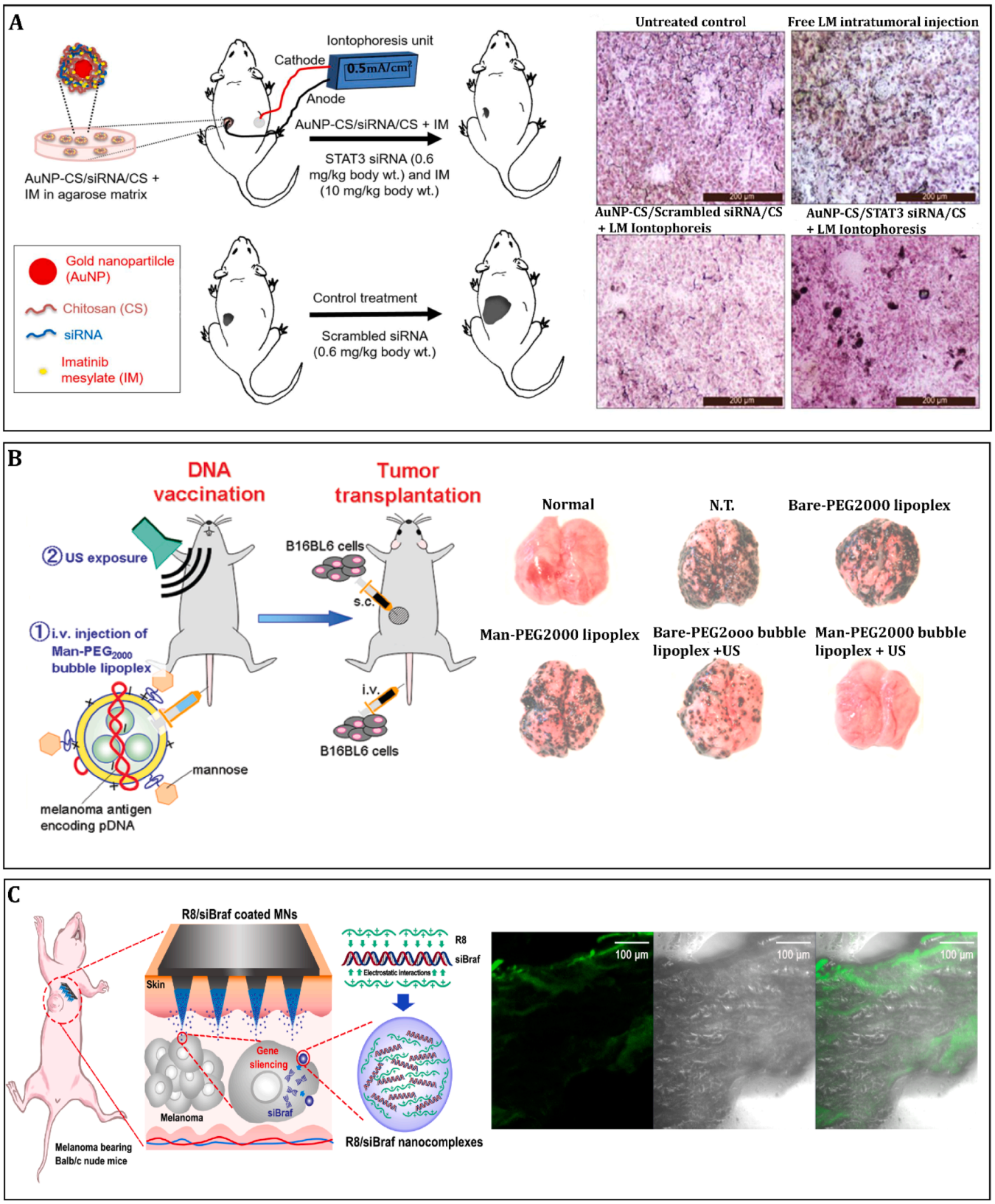

Fig. 5. Faithful physical gene delivery approaches for melanoma therapy: (A) Electroporation technique, which evaluated the expression of STAT3 protein in tumor bearing C57BL/6 mouse, Reproduced with permission from ref (Labala et al., 2017) Copyright 2017 Elsevier. **(B)** Sonoporation technique, which used a DNA vaccination for metastatic and relapsed melanoma by ultrasound based on polymeric-based nanovectors. Reproduced with permission from ref (Un et al., 2011) Copyright 2011 American Chemical Society. **(C)** Microneedle technique, which used siRNA delivery system based on cell penetrating peptide octa arginine (R8) nanocomplexes combined with coated microneedles., Reproduced with permission from ref (Ruan et al., 2018) Copyright 2018 Elsevier.

Conceptualization; Supervision; Writing - review & editing, S-H.-J.: Funding acquisition; Project administration, M.C.: Supervision; Writing - review & editing. All authors have read and agreed to the published version of the manuscript.

Funding

S.H.J. is deeply grateful to Isfahan University of Medical Sciences, Deputy of Research for their research support (Grant No. 198142). M.C. was supported with a Ramon y Cajal contract from the Spanish Ministry of Science and Innovation, "Agencia Estatal de Investigación" (MCIN/AEI/10.13039/501100011033), and European UnionNextGeneration (EU/PRTR), funding reference: RYC2021-031003-I. M.C. was also funded by Maria Zambrano contract from Spanish Ministry of Universities, European Union NextGeneration and Complutense University of Madrid.

Declaration of Competing Interest

There is no conflict of statement.

Data availability

No data was used for the research described in the article.

Acknowledgments

We gratefully thank the staff of applied physiology research center for their support.

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