MOLEKÜLER BİYOLOJİ VE GENETİK ALANINDA BİLİMSEL ARAŞTIRMALAR

Editör: Doç.Dr. Meryem Şenay ŞENGÜL DEMİRAK

yaz
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E_ISBN 978-625-8574-54-8

Mart 2026 – Afyonkarahisar

Dizgi/Mizanpaj: YAZ Yayınları

Kapak Tasarım: YAZ Yayınları

YAZ Yayınları. Yayıncı Sertifika No: 73086

M.İhtisas OSB Mah. 4A Cad. No:3/3
İscehisar/AFYONKARAHİSAR

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İÇİNDEKİLER

MELANOMDA APOPTOTİK YOLAKLARI HEDEFLEYEN BAKTERİ KÖKENLİ DOĞAL BİLEŞİKLER: MOLEKÜLER MEKANİZMALAR VE TERAPÖTİK ÇIKARIMLAR

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1. GİRİŞ

Deri (cilt) kanserleri, dünya genelinde giderek artan önemli bir halk sağlığı sorunu olup son 70 yıl içerisinde deri kanseri görülme sıklığının yaklaşık 50 kat arttığı bildirilmektedir (Park vd., 2020). Bu artış, ultraviyole (UV) radyasyona maruziyetin artması, nüfusun yaşlanması, koruyucu önlemlerin yetersiz kalması gibi çok sayıda çevresel ve demografik faktörle ilişkilendirilmektedir (Wang vd., 2025).

Deri kanserleri, iki ana gruba ayrılmaktadır: malign (kötü huylu) melanom ve melanom dışı deri kanserleri. Bu iki grup arasındaki temel farklılıklar, hücresel köken, büyüme hızı, invazyon kapasitesi ve metastaz potansiyeli gibi klinik özelliklere dayanmaktadır (Elder vd., 2020). Melanomun deri yüzeyinde ortaya çıkan (kutanöz) tiplerinin yanı sıra, avuç içi, ayak tabanı, el-ayak parmakları, tırnak altı gibi özel bölgelerde, ağız ve burun boşluğu gibi mukozal yapılarda veya gözde ortaya çıkan tipleri

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tanımlanmış olup, en yaygın moleküler alt tipleri BRAF, NRAS veya NF1 genlerinde mutasyonlar taşımaktadır (Fateeva vd., 2024). Yüksek invazyon kapasitesi ve belirgin metastik eğilimi nedeniyle melanom, deri kanserleri arasında en ölümcül form olarak kabul edilmektedir. Erken evrede tanı konulmadığı takdirde melanom hücreleri, lenfatik ve hematojen yollarla hızlı yayılım gösterme eğilimindedir; bu güçlü agresiflik özelliği, melanomu en tehlikeli deri kanseri formu hâline getirmektedir (Cives vd., 2020; Nanz vd., 2024).

Dünya Sağlık Örgütü tarafından yayımlanmış kanser istatistiklerine göre, 2022 yılında dünya çapında yaklaşık 330.000 yeni melanom vakası tanımlanmış ve 60.000'e yakın kişi bu hastalık nedeniyle yaşamını yitirmiştir (WHO, 2022). Aynı dönemde melanom dışı deri kanserleri için bildirilen yeni vaka sayısı bir milyonun üzerinde olup, bu veriler melanomun nispeten daha düşük insidansa sahip olmasına rağmen deri kanserine bağlı ölümlerin büyük bir bölümünden sorumlu olduğunu göstermektedir (Wang vd., 2025; Langselius vd., 2025). Geleceğe ilişkin epidemiyolojik kestirimler de pek parlak gözükmemektedir. Önlemler ve tedaviler yetersiz kalırsa, melanomdan kaynaklanan küresel yükün 2040 yılına kadar 510.000 yeni vakaya ve 96.000 ölüme ulaşacağı tahmin edilmektedir (Arnold vd., 2022).

Melanom insidansının yaşla birlikte belirgin şekilde arttığı, özellikle açık tenli bireylerde ve ileri yaş grubunda melanom riskinin daha yüksek olduğu bildirilmektedir. Güneş ışığına maruziyet, deri kanserlerinin en önemli risk faktörüdür. Özellikle çocukluk ve ergenlik dönemlerinde meydana gelen şiddetli güneş yanıkları, ilerleyen yaşlarda cilt yüzeyinde ortaya çıkan (kutanöz) melanom gelişme riskini anlamlı derecede artırmaktadır (Oliveria vd., 2006; Raimondi vd., 2020; Feng vd., 2025). Melanom gelişimi yalnızca genetik yatkınlık, deri tipi ve bağışıklık durumu gibi bireysel faktörlerle sınırlı olmayıp;

eğitimsizlik, gelir düzeyi ve sağlık hizmetlerine erişim olanakları, yaşam koşulları gibi sosyoekonomik faktörlerden de belirgin biçimde etkilenmektedir (Raimondi vd., 2020; Lal vd., 2025).

Coğrafi dağılım açısından değerlendirildiğinde, melanom insidansının dünya genelinde belirgin bir bölgesel farklılık gösterdiği görülmektedir. Özellikle Avustralya ve Yeni Zelanda, yaşa göre standardize edilmiş insidans oranlarının en yüksek olduğu ülkeler olarak öne çıkmaktadır. Avrupa ve Kuzey Amerika da melanom insidansının yüksek olduğu bölgeler arasında yer almakta; buna karşın Afrika ve Asya kıtalarında insidansın belirgin ölçüde daha düşük olduğu bildirilmektedir (Arnold vd., 2022; Wang vd., 2025; Roky vd., 2025).

Melanomun tedavisinde, hastalığın tipine ve evresine göre cerrahi, radyoterapi ve kemoterapi gibi konvansiyonel yaklaşımların yanı sıra, son yıllarda hedefe yönelik terapi ve immünoterapi gibi seçeneklerle önemli ilerlemeler kaydedilmiş olsa da, ileri evre melanomların tedavisinde, kullanılan ajanların ciddi yan etkileri, tedavi direnci, hastalık nüksü ve metastaz gibi klinik açıdan önemli sorunlarla karşılaşılabilmektedir (Patel vd., 2021; Fateeva vd., 2024). Tedaviye direncin altında yatan temel biyolojik mekanizmalardan biri, melanomun programlanmış hücre ölümü olan apoptozdan kaçabilme yeteneğidir. Apoptoz, kanser hücrelerinin kontrolsüz proliferasyonunu engelleyen temel bir mekanizmadır ve bu süreçten kaçış tümör oluşumunu destekleyerek tedavi direncine yol açar. Bu nedenle modern kanser terapilerinin temel hedefi, normal hücrelere minimal zarar verirken kanser hücrelerinde apoptozu seçici olarak indüklemektir. Apoptotik yolakları hedefleyen tedavi stratejileri, gen tedavisi ve immünoterapi alanlarında da önemli bir araştırma konusu olup inflamasyonu sınırlayarak programlı hücre ölümünü artırma potansiyeli taşımaktadır (An vd., 2025). Melanom hücrelerinde anti-apoptotik proteinlerin aşırı ekspresyonu, kaspaz aktivasyonunun baskılanması, p53 fonksiyon bozuklukları ve

PI3K/AKT ile MAPK gibi hücre sağkalım yolaklarının sürekli aktivasyonu apoptoz direncinin gelişimine katkıda bulunduğundan, tedavi yaklaşımları genelde bu süreçleri tersine döndürme yönünde planlanmaktadır (Hussein vd., 2003; Patel vd., 2021; Fateeva vd., 2024).

Melanomun gelişiminde rol oynayan metabolik süreçler, baskın sinyal yolakları ve mevcut ilaçların moleküler hedefleri güncel bir derlemede ayrıntılı şekilde ele alınmıştır (Patel vd., 2021). Hedefli terapötik yaklaşımlar daha çok hücre çoğalmasının ve hücre sağkalımının azaltılmasında etkili çeşitli inhibitörleri içermektedir; hedefler arasında B-Raf serin/treonin kinaz (BRAF), mitojenle aktive olan protein kinaz (MEK), mikroftalmi ile ilişkili transkripsiyon faktörü (MITF), reseptör-tirozin kinaz MET (c-Met), reseptör-tirozin kinaz Kit (c-KIT), vasküler endotelyal büyüme faktörü reseptörü (VEGFR) ve fosfoinositid-3-kinaz AKT sinyal yolu (PI3K-AKT) yer almaktadır (Patel vd., 2021).

Son yıllarda doğal kaynaklardan elde edilen antikanser bileşikler, düşük toksisiteleri, çoklu moleküler hedefleri aynı zamanda modüle etme kapasiteleri ve mevcut tedavi yaklaşımlarına duyarlılığı artırabilme potansiyelleri nedeniyle melanom tedavisinde potansiyel terapötik veya yardımcı ajanlar olarak dikkat çekmektedir (Chinembiri vd., 2014; Gao vd., 2025). Bu bileşiklerin birçoğunun melanom hücrelerinde apoptoz, mitokondriyal disfonksiyon, kaspaz aktivasyonu, ROS (reaktif oksijen türleri) aracılı ölüm sinyallerinin tetiklenmesi ve endoplazmik retikulum stresine bağlı programlı hücre ölümü gibi çoklu mekanizmalarla antitümör etkiler gösterdiği bildirilmiştir (Gao vd., 2025; An vd., 2025). Özellikle Resveratrol, Nisin Z gibi bileşiklerin apoptoz yolaklarını aktive ettiği ve melanoma proliferasyonunu baskıladığı gösterilmiştir (Gao vd., 2025).

Bu bölümde, apoptotik yolaklar üzerinden anti-melanom aktivitesi gösteren bakteri kaynaklı bileşiklerden önemli örnekler sunulmaktadır. Amacımız, bu bileşiklerin terapötik potansiyellerini özetlemenin ötesine geçerek etki mekanizmalarını vurgulamak ve literatürdeki bilgi boşluklarına dikkat çekmektir. Etki mekanizmaları özellikle apoptotik yolaklarla ilişkilendirilen peptid ve protein yapısındaki moleküller bağlamında tartışılmıştır. Ayrıca, klinik uygulamaya geçişte karşılaşılan zorluklar ele alınmıştır. Sunulan güncel bilgiler, PubMed ve Scopus veritabanlarında 2000–2026 yılları arasında yayınlanmış konu ile ilgili bilimsel makalelere, global otoritelerin bildirimlerine ve temel kaynaklara dayanmaktadır.

2. MELANOMDA APOPTOTİK YOLAKLARI HEDEFLEYEN BAKTERİ KAYNAKLI ANTİKANSER BİLEŞİKLER

Son yıllarda, bakteri kaynaklı antikanser bileşiklerin melanom hücrelerinde baskılanmış apoptotik yolakları yeniden aktive etme potansiyeli, kanser biyolojisi ve translasyonel onkoloji alanlarında giderek artan bir ilgi odağı hâline gelmiştir (Felgner vd., 2016).

Günümüzde kanserlerin bakteriyel yaklaşımlar ile tedavisi, canlı, atenüe veya genetik olarak modifiye edilmiş bakterilerin yanı sıra bakteri kaynaklı biyomolekülleri kapsamaktadır. Bakteri kaynaklı bileşiklerin mitokondriyal apoptotik yolakları aktive ederek kaspaz kaskadını tetiklediği, anti-apoptotik proteinlerin (özellikle Bcl-2 ailesi üyeleri) ekspresyonunu baskıladığı ve ölüm reseptörü aracılı ekstrinsik apoptotik sinyalleri güçlendirdiği gösterilmiştir. Ayrıca, bu ajanların tek başına uygulandıklarında tümör büyümesini inhibe edebildiği, konvansiyonel kemoterapötik veya immünoterapötik yaklaşımlarla kombine edildiklerinde ise terapötik etkinliği

artırabildiği bildirilmektedir (Felgner vd., 2016; Khan vd., 2024; Pyla vd., 2025).

Bu bölümde, melanom tedavisinde umut vadeden bakteri kökenli antikanser bileşikler ve bu bileşiklerin apoptotik ve anti-apoptotik mekanizmalar üzerindeki etkileri yapı temelli ve sistematik yaklaşımlarla ele alınmaktadır.

2.1. Bakteriyel Peptidler

Bakteriyel peptidler, melanom hücrelerinde programlı hücre ölümünü indükleyebilme kapasiteleri nedeniyle son yıllarda yoğun biçimde araştırılmaktadır. Melanom hücrelerinde apoptotik hücre ölümünü tetikleyebilen peptidler arasında, çoğunlukla laktik asit bakterileri ve bazı Gram-negatif türler tarafından üretilen ribozomal kökenli katyonik peptidler olan **bakteriyosinler** dikkati çekmektedir. Mevcut kanıtlar, bakteriyosinlerin kanser hücrelerinin zarlarındaki negatif yüklü bileşenlerle seçici etkileşim kurabildiğini ve bunu takiben mitokondriyal disfonksiyon/oksidatif stres üzerinden intrinsik apoptoz sinyallemesini aktive edebildiğini göstermektedir. Bu süreçte Bax/Bcl-2 dengesinin pro-apoptotik yönde değişmesi, mitokondriyal membran potansiyel kaybı, sitokrom *c* salınımı ve kaspaz-9 → kaspaz-3 aktivasyonu gibi basamakların sıklıkla rapor edildiği; ayrıca bazı bakteriyosinlerin kaspaz-8 inhibitörü c-FLIP'in ekspresyonunu baskılayarak ekstrinsik yolak üzerinden de apoptozu teşvik edebildiği bildirilmektedir (Wang vd., 2024b). Melanom bağlamında en iyi karakterize örneklerden biri nisin Z'dir (Lewies vd., 2018). *Lactococcus lactis* tarafından üretilen nisin Z'nin kültüre edilmiş insan melanom hücrelerinde (A375) malign olmayan keratinositlere kıyasla daha belirgin sitotoksisite oluşturduğu, melanom hücre metabolizmasını baskılayarak ROS üretimini artırdığı ve sonuçta apoptotik hücre ölümünü indüklediği gösterilmiştir (Lewies vd., 2018). Daha güncel bir çalışmada, nisin Z'nin B16F10 melanom hücrelerinde oksidatif

stres artışı, mitokondriyal disfonksiyon ve kaspaz-3 aracılı apoptoz ile ilişkili olduğu; bu etkinin nekrotik hücre ölümünden ziyade düzenlenmiş apoptotik mekanizmalar üzerinden gerçekleştiği belirlenmiştir (Monfared vd., 2023). Literatürde nanoforma getirilmiş nisin ile melanom hücrelerinde yürütülmüş bir çalışma da yer almaktadır. Nisin yüklü polikaprolakton/serisin nanoliflerin G361 melanom hücrelerinde apoptotik ve proliferatif yolaklarla ilişkili genlerin (örneğin, kaspaz-3, Bax/Bcl-2, TRAIL-1/2, Bcl-xL, Silkin D1) ekspresyonunu değiştirerek apoptozu desteklediği ve aynı zamanda oksidatif stres ve enflamasyon belirteçlerini modüle ettiği rapor edilmiştir (Erdoğmuş vd., 2026). Bu bulgular, özellikle nisin gibi bakteriyosinlerin melanomda apoptotik yolakları tetikleyebilme potansiyeline sahip olduğunu göstermektedir. Bununla birlikte, nanotaşıyıcı sistemler ve yapısal optimizasyon yaklaşımlarıyla bu tip bileşiklerin moleküler stabilitesinin, biyoyaralanımının ve hedefe yöneliminin artırılabileceği vurgulanmaktadır (Wang vd., 2024b; Erdoğmuş vd., 2026).

Melanom hücrelerinde apoptozu indükleyen başka bakteriyel peptidler de vardır. *Bacillus* türlerinden izole edilen siklik lipopeptidlerden sürfaktinin B16F10 melanom hücre hattında hücre canlılığını anlamlı düzeyde azaltarak apoptotik hücre ölümünü tetiklediği; bu etkinin mitokondriyal membran potansiyeli kaybı, sitokrom *c* salınımı ve kaspaz-9/kaspaz-3 aktivasyonu ile ilişkili olduğu rapor edilmiştir (Kim vd., 2021). Benzer şekilde *Pseudomonas fluorescens* kaynaklı bir lipopeptid olan psödofaktin II, melanom hücrelerinde Annexin V pozitifliğinde artışa, DNA fragmantasyonuna ve ROS üretimi ile karakterize intrinsik apoptotik yolak aktivasyonuna yol açarken, normal fibroblast hücrelerine karşı gösterdiği daha düşük sitotoksisite ile seçici bir etki profili sergilemiştir (Janek vd., 2013).

Literatürde antikanser proteinlerin belli parçalarından oluşan peptidlerin (örn. p28) de ana molekülü olan proteinler (örn. azurin) gibi, melanom hücrelerinde apoptozu tetiklediği gösterilmiştir (Yaghoubi vd., 2020).

2.2. Bakteriyel Proteinler ve Enzimler

Melanom hücrelerinde proliferasyonun baskılanması ve programlı hücre ölümünün indüklenmesi açısından dikkat çeken bakteri kaynaklı antikanser ajanlar arasında bakteriyel proteinler ve enzimler de yer almaktadır. Bu moleküller çoğunlukla hücre içi sinyal yolaklarını hedefleyerek apoptotik mekanizmaları aktive edebilmektedir. Özellikle *Pseudomonas aeruginosa* kökenli bakır bağlayıcı bir redoks proteini olan azurin, melanom hücrelerinde tümör baskılayıcı p53 proteininin stabilizasyonunu artırarak hücre döngüsü duraksamasına ve intrinsik apoptotik yolak aktivasyonuna yol açmaktadır; bu süreçte kaspaz-9 ve kaspaz-3 aktivasyonu ile mitokondriyal disfonksiyonun rol oynadığı bildirilmiştir (Yamada vd., 2002). Benzer şekilde, streptokok ve aktinomisetler dahil bakterilerde (özellikle *Mycoplasma* türlerinde) yaygın olan enzimlerden biri olan arjinin deiminazın (ADI), melanom hücrelerinin arjinin bağımlı metabolizmasını hedefleyerek amino asit yoksunluğuna neden olduğu ve bu metabolik stresin mitokondriyal membranda potansiyel kaybına, sitokrom *c* salınımına ve kaspaz aracılı apoptoza yol açtığı bildirilmektedir (Wu vd., 2021; Field vd., 2023).

2.3. Bakteriyel Toksinler

Bakteriyel toksinler ve toksin türevleri, diğer bazı kanserlerde olduğu gibi melanomda da (özellikle MAPK/ERK yoluna bağımlı alt tiplerde), apoptotik hücre ölümünü tetikleyebilmektedir (Misra vd., 2025). *Bacillus anthracis* tarafından salgılanan bir virülans faktörü olan şarbon ölümcül toksini (LT) ve çeşitli türevlerinin, mitojenle aktive olan protein

kinaz kinazlara (MEK'ler) yönelik proteolitik aktiviteleri nedeniyle, mitojenle aktive olan protein kinaz (MAPK) yolaklarını (ERK, p38, JNK) etkili bir şekilde bloke ederek apoptoz direncini kırdığı ve BRAF V600E aktive edici mutasyona sahip insan melanomlarına seçici olarak toksik olduğu bulunmuştur (Liu vd., 2008). Bakteri toksinlerinin melanom hücrelerine karşı etkinliğini ve özgüllüğünü artırmak amacıyla MEK yönlendirmeli farklı LT'ler ve rekombinant türevleri olan kimerik toksinler de geliştirilmiş ve preklinik çerçevede değerlendirilmiştir (Koo vd., 2002; Khoshnood vd., 2022).

Ayrıca, malign melanom ve melanom hücre hatlarının çoğunda ifade edilen bir yüzey antijeni olan HMW-MAA'yı tanıyan 9.2.27 antikoruna kimyasal olarak bağlanmış Pseudomonas ekzotoksin A (9.2.27PE) immünotoksininin, melanoma hücrelerinde protein sentezini baskılayarak apoptotik özellikler gösteren hücre ölümüne yol açtığı gösterilmiştir (Risberg vd., 2009).

Son olarak, *Clostridium perfringens* enterotoksininin (CPE) klaudin reseptörlerine bağlanıp membranda por oluşturarak hızla sitotoksisiteye yol açtığı raporlanmıştır (Rathnayake vd., 2024; Nagarajan vd., 2025). Son yıllarda yürütülmüş bu yapısal çalışmalarda, CPE-klaudin etkileşiminin moleküler temeli ayrıntılı şekilde ortaya konulmuş, melanomda uygulanabilirliğin tümörün klaudin ekspresyon profiline bağlı olduğu belirlenmiş, bu da klaudin ekspresyonu yüksek olan tümörlerde hedefli kullanım yaklaşımını gündeme getirmiştir.

2.4. Diğer Bakteriyel Metabolitler ve Polisakkaridler

Melanomda apoptotik programın yeniden etkinleştirilmesini sağlayan bakteriyel sekonder metabolitlerden biri salinosporamid A (marizomib) isimli küçük moleküldür. Denizel aktinomiset *Salinispora tropica* kaynaklı bu 20S proteazom inhibitörünün A375 ve G361 insan melanom

hücrelerinde proteostatik stres ve endoplazmik retikulum stresiyle birlikte apoptotik yanıtı artırdığı gösterilmiştir (Piskorz vd., 2024). Benzer şekilde, *Serratia marcescens* tarafından üretilen prodigiosin isimli pigmentin SK-MEL-5 melanom hücrelerinde anti-apoptotik MCL-1/BAK komplekslerini bozarak sitokrom *c* salınımı ve kaspaz aktivasyonu aracılığıyla mitokondriyal apoptotik yolu aktive ettiği bulunmuştur (Hosseini vd., 2013). Bunlara ek olarak, prodigiosin adezyonu, filopod oluşumunu ve metastatik davranışı baskılamaktadır (Espona-Fiedler vd., 2022).

Lactiplantibacillus plantarum WLPL09 suşundan izole edilen bir ekzo-polisakkaridin (EPS-09) B16F10 fare melanom modelinde anlamlı bir antitümör aktivite gösterdiği saptanmıştır (Wang vd., 2024a). Moleküler düzeyde yapılan analizler, EPS-09 uygulamasının p53, kaspaz-3 ve kaspaz-9 gibi apoptoz ile ilişkili genlerin ekspresyonunu ve Bax/Bcl-2 oranını artırdığını; buna karşılık VEGF ve FGF2 gibi anjiyogenez belirteçlerinin transkripsiyonunu baskıladığını göstermiştir. Buradan da anlaşılacağı üzere, bakteriler peptid/protein yapısı dışında başka anti-melanom bileşikler de üretilebilmektedir.

3. GENEL DEĞERLENDİRME VE GELECEK PERSPEKTİFLERİ

Melanom, kanser hücrelerinde ortaya çıkan apoptozdan kaçış mekanizmaları sayesinde, özellikle ileri evrelerde belirgin invazyon ve metastaz potansiyeli sergileyerek tedaviye direnç geliştirebilen bir malignitedir. Bu bağlamda intrinsik (mitokondriyal) ve ekstrinsik (ölüm reseptörü aracılı) apoptotik yolakların yeniden etkinleştirilmesi; Bcl-2 ailesi, IAP proteinleri, c-FLIP, p53 düzenleyicileri ve MAPK/PI3K-AKT gibi sağkalım sinyalleri ile ilişkili direnç düğümlerinin hedeflenmesi, terapötik açıdan rasyonel stratejiler sunmaktadır.

Bu bölümde özetlenen bilgiler, başta peptidler olmak üzere bakteri kökenli doğal antikanser bileşiklerin melanom hücrelerinde baskılanmış apoptotik programı yeniden devreye sokabildiğini göstermektedir. Bakteriyosinler dahil bazı bakteriyel peptidler, membran etkileşimi, oksidatif stres artışı ve mitokondriyal disfonksiyon üzerinden kaspaz kaskadını aktive edebilmekte, bazıları ise ölüm reseptörü sinyallerini güçlendirerek ekstrinsik yolağın katkısını artırabilmektedir. Bakteriyel proteinler/enzimler ve toksin/toksin türevleri ise p53 stabilizasyonu, metabolik stres (örn. arjinin yoksunluğu), proteazom inhibisyonu ve MAPK sinyalinin kesilmesi gibi farklı biyolojik eksenler üzerinden apoptotik eşiği düşürme potansiyeli taşımaktadır.

Bu çeşitlilik, bakteri kökenli bileşiklerin tek hedefli ajanlardan farklı olarak "çoklu hedef" profili ile melanomun adaptif direnç kapasitesini sınırlayabilecek adaylar olabileceğine işaret etmektedir.

Bununla birlikte, bakteri kökenli doğal bileşiklerin gösterdiği anti-melanom etkilerin *in vivo* çalışmalarla doğrulanması ve terapötik açıdan ayrıntılı biçimde değerlendirilmesi klinik uygulamalar için kritik öneme sahiptir. Preklinik düzeyde umut vadeden bu ajanların klinik uygulamaya taşınmasında aşılması gereken kritik eşikler bulunmaktadır. İlk olarak, bakteri kökenli birçok bileşik için seçicilik penceresi (terapötik indeks), normal hücreler üzerindeki olası toksisite ve immünojenisite riski hâlen yeterince çalışılmamıştır. İkinci olarak, *in vitro* apoptotik yanıtların, tümör mikroçevresi, hipoksi, bağışıklık baskılanması ve heterojen klonal yapı gibi *in vivo* faktörlerin varlığında sürdürülebilirliği net değildir. Üçüncü olarak, peptid/protein tabanlı ajanlarda proteolitik yıkım, kısa yarı ömür, hedef dokuya etkin ulaşım ve biyoyararlanım gibi klinik başarıyı belirleyen başlıca farmakokinetik değişkenlerin yeterince incelenmediği görülmektedir.

Bu nedenle, bakteri kökenli anti-melanom ajanların *in vivo* etkinliklerinin, immünojenisite potansiyellerinin ve farmakokinetik özelliklerinin daha ayrıntılı biçimde değerlendirilmesi gerekmektedir. Dolayısıyla bu ajanların kliniğe yansıyabilmesi, mekanizma odaklı biyobelirteçlerle (kaspaz aktivasyonu, MOMP göstergeleri, Bax/Bcl-2 dengesi, IAP/c-FLIP düzeyleri, p53 aktivasyon imzaları vb.) ispatlanmış biyolojik etkinliğin yanı sıra, uygun taşıma ve formülasyon stratejilerine bağlıdır.

Sonuç olarak, bakteri kökenli doğal bileşikler melanomda apoptotik yolakların yeniden etkinleştirilmesi için güçlü bir biyolojik temel sunmakta ve çoklu hedef profilleri nedeniyle direnç dinamiklerini aşmada potansiyel avantajlar taşımaktadır. Eşzamanlı olarak yürütülecek mekanizma temelli *in vitro* ve *in vivo* aktivite ve güvenlik testleri, farmakokinetik/formülasyon çalışmaları, rasyonel kombinasyon ve nanoform tasarımları ve fizibilite değerlendirmeleri, bakteri kökenli ajanların melanom tedavisinde tamamlayıcı ya da yeni nesil terapötik adaylar olarak konumlandırılmasını destekleyecek ve klinik translasyonu hızlandıracak bir yol haritası sunmaktadır.

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CRISPR-CAS9 GENOME EDITING: FROM MOLECULAR MECHANISMS TO CLINICAL TRANSLATION AND ETHICAL CHALLENGES

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1. INTRODUCTION

Significant advancements have been made in recent years regarding the application of Clustered Regularly Interspaced Palindromic Repeats (CRISPR) and CRISPR-associated protein (Cas) systems in genetic engineering. Several variants of CRISPR-Cas9 systems are being used as genome editing tools. Recently, some CRISPR-Cas systems without genome editing capabilities were introduced. These CRISPR-Cas systems are classified into Class I and Class II systems. Currently, various research groups and companies are in competition to secure patents for these systems as many powerful applications are being developed simultaneously (Perez Rojo et al., 2018).

The CRISPR/Cas system, which is an abbreviation for Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein, has significantly influenced the field of genetic engineering through its ability to increase efficiency in genome editing. The CRISPR system was first obtained from bacteria and archaea that employ it as a protective mechanism against nucleic acids of viruses including bacteriophages or plasmids (Hryhorowicz et al., 2023; Ishino et al., 1987). The CRISPR system comprises three major processes: spacer acquisition, CRISPR RNA (crRNA) synthesis and

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processing, and interference against nucleic acids of viruses (Hryhorowicz et al., 2017).

In the case of the CRISPR/Cas9 system of type II CRISPR/Cas, a DNA-cutting enzyme called Cas9 is guided by a molecule called CRISPR RNA (crRNA) as well as a molecule called trans-activating RNA (tracrRNA) to target DNA from viruses. In 2012, Doudna and Charpentier demonstrated that by using Cas9 along with crRNA and tracrRNA, it was possible to introduce a double strand break into a given DNA sequence. This discovery was recognized by the Chemistry Nobel Prize in 2020. Although other DNA nucleases are able to perform genome editing, it is only through the simplicity, cost-effectiveness, accuracy, and efficiency of the CRISPR/Cas system that has made it a widely accepted tool for DNA manipulation.

Scope and Contribution of This Chapter

This chapter provides a comprehensive and integrative overview of CRISPR-Cas9 genome editing technology, spanning its molecular foundations, technological advancements, clinical applications, and ethical implications. Unlike narrowly focused reviews, this contribution contextualizes CRISPR-based approaches within translational medicine, emphasizing both therapeutic potential and societal challenges. By synthesizing experimental, clinical, and ethical perspectives, this chapter aims to serve as a reference framework for researchers, clinicians, and policymakers engaged in genome editing research and its responsible implementation.

2. CRISPR/Cas9

Of all the CRISPR/Cas systems identified so far, the type II (Class II) system is the most commonly used for genetic engineering due to its ease of use, flexibility, and efficiency

(Hryhorowicz et al., 2017). In the laboratory, the CRISPR/Cas9 system is based on two components: a single guide RNA (sgRNA) and the Cas9 enzyme. The sgRNA is a short synthetic RNA made up of 20 nucleotides derived from the tracrRNA and crRNA. The target DNA sequence is unique to the genome and must be located adjacent to a PAM site; for SpCas9 derived from *S. pyogenes*, the PAM site is 5'-NGG-3'.

The Cas9 protein, with the aid of an RNA guide, contains two active nuclease site regions: HNH and RuvC. The Cas9 protein can cut the desired target site in the genome. Using the Cas9 protein with two or more unique sgRNA can edit the genome to an extent where you can edit multiple genes or even remove parts of the genome (Cong et al., 2013). However, SpCas9 can have up to one nucleotide difference between the target site and the guide RNA. This can lead to off-target effects, which can be dangerous (Hsu et al., 2013; Zhang et al., 2015). To solve this issue, scientists are trying to improve Cas9 to increase the target site specificity for safer genome editing. A number of precise Cas9 variants have been created (Kleinstiver et al., 2016; Vakulskas et al., 2018; Casini et al., 2018; Slaymaker et al., 2016; Chen et al., 2017; Lee et al., 2018; Hu et al., 2018; Allemailem et al., 2023). The second limitation is the presence of PAMs. To solve this limitation, researchers have found Cas9 orthologs with a broader range of PAMs (Ran et al., 2015; Kim et al., 2017; Müller et al., 2016; Hou et al., 2013; Nishimasu et al., 2018; Walton et al., 2020; Kleinstiver et al., 2015). This increases the genome editing range.

3. APPLICATIONS

The CRISPR/Cas system has provided a whole new realm of possibilities to target specific genes. It has shown promise in the treatment of human diseases, diagnostics, and the

advancement of biotechnology. When referring to the application of the CRISPR/Cas9 system in the context of the clinic, the therapies can be divided into two broad categories: ex vivo and in vivo.

For the ex vivo approach, the patient's cells are taken, edited, and reintroduced into the body. This approach has been considered in the context of cancer immunotherapy, monogenic diseases, and antiviral therapies. An example of the successful application of the CRISPR/Cas9 system is the editing of T cells to target immune checkpoints or TCR-related genes. Another example is the editing of the erythroid-specific enhancer of the BCL11A gene in hematopoietic stem/progenitor cells. This approach has shown therapeutic benefits for blood disorders such as sickle cell anemia or β-thalassemia. Aside from the aforementioned examples, the CRISPR/Cas9 system is being considered to treat other diseases such as cystic fibrosis or Duchenne muscular dystrophy.

The CRISPR/Cas9 system is being considered to treat infectious diseases such as HIV by editing the host entry factors such as the CCR5 gene or the virus genome. During the COVID pandemic, novel concepts of editing immune cells have been evaluated in the clinic.

The first application of the CRISPR/Cas9 system to treat a disease in humans was the editing of the CEP290 gene to treat Leber's congenital amaurosis 10 (LCA10) via a subretinal injection approach. This marked the beginning of the local application of the CRISPR/Cas9 system to treat a disease. This demonstrates the importance of monitoring the safety of the system to treat diseases.

3.1. Base Editing and Prime Editing as Next-Generation Extensions

Genome editing capabilities have been expanded by base editing and prime editing. Cytosine base editors (CBEs) (Komor, Kim, Packer, Zuris, & Liu, 2016) and adenine base editors (ABEs) (Gaudelli et al., 2017) enable programmable nucleotide conversions without inducing double-strand breaks, often reducing indel formation. Prime editing further broadens editing scope to include multiple substitution types as well as insertions and deletions with improved precision (Anzalone et al., 2019). Together, these platforms represent a major step toward safer and more versatile therapeutic genome engineering.

Table 1. Representative clinical and experimental applications of CRISPR/Cas9 genome editing in human diseases. Clinical and experimental applications of CRISPR/Cas9 genome editing in human diseases. Representative clinical and preclinical applications include cancer immunotherapy, hereditary blood disorders, infectious diseases (HIV, COVID-19 concepts), and in vivo ocular delivery for *LCA10*.

Disease/Indication	Therapeutic Setting	Clinical/Experimental Use	Delivery / Platform	Development Stage	Target (Gene) and Edited Cell/Tissue
Metastatic non–small-cell lung cancer	Ex vivo	Cancer immunotherapy (engineered T cells)	Electroporation of CRISPR components into patient-derived T cells	Phase 1 (ClinicalTrials.gov: NCT03399448)*	PDCD1 (PD-1) knockout and/or TCR pathway components (e.g., TRAC/TRBC) in T cells
Sickle cell disease, β-thalassemia	Ex vivo	Reactivation of fetal hemoglobin (HbF) via enhancer disruption	Electroporation into HSPCs, followed by autologous infusion	Phase 2/3 (ClinicalTrials.gov: NCT03655678)*	BCL11A erythroid enhancer disruption in HSPCs

HIV-1 infection (AIDS)	Ex vivo	Host entry factor editing to reduce viral susceptibility	HSPC editing and transplantation	Clinical / early translational (ClinicalTrials.gov: NCT03164135)*	CCR5 knockout in HSPCs (host cells)
HIV-1 infection (AIDS)	In vivo (preclinical)	Proviral DNA targeting/excision	Systemic delivery in animal models (construct-dependent)	Preclinical (mouse model)	Multiplex targeting of HIV-1 proviral sequences in infected tissues
COVID-19 (conceptual immune-cell editing)	Ex vivo	Exploratory immune modulation for long-term protection	Ex vivo gene-edited T cells	Phase 1/2 (ClinicalTrials.gov: NCT04990557)*	PDCD1 (PD-1) and ACE2 knockout in T cells
Leber's congenital amaurosis 10 (LCA10)	In vivo	Local ocular genome editing	Subretinal/ local injection near photoreceptor cells	First-in-human localized in vivo approach (2020 milestone)	CEP290 targeting in retinal tissue (photoreceptor region)

AAV, adeno-associated virus; ACE2, angiotensin-converting enzyme 2; HbF, fetal hemoglobin; HIV, human immunodeficiency virus; HSPC, hematopoietic stem/progenitor cell; LCA10, Leber's congenital amaurosis 10; NSCLC, non–small-cell lung cancer; PD-1 (PDCD1), programmed cell death protein 1; TCR, T-cell receptor.

4. CRISPR/Cas9 IN TUMOR RESEARCH AND TRANSLATIONAL ONCOLOGY

CRISPR-Cas9 has become a foundational tool in oncology research by enabling systematic interrogation of oncogenic drivers, tumor suppressor pathways, drug resistance mechanisms, and synthetic lethal interactions. In tumor models, CRISPR-based gene perturbation supports mechanistic studies, target discovery, and functional screening, frequently complemented by stem cell and organoid platforms. These approaches have accelerated the identification of actionable

vulnerabilities and helped prioritize targets for precision oncology strategies (Augert et al., 2020; Wang et al., 2013; Bayarsaikhan, Bayarsaikhan, & Lee, 2021; Ledford, 2019; Wang et al., 2018; Horii et al., 2013; Paquet et al., 2016; Xie et al., 2014; Liu, Zhang, Liu, & Cheng, 2017; Drost et al., 2015).

4.1. Breast Cancer

In breast cancer, CRISPR/Cas9 has been used to dissect subtype-specific dependencies, investigate endocrine resistance, and identify regulators of proliferation, invasion, and therapy response. Functional studies and screening approaches have supported target validation and improved understanding of heterogeneity across molecular subtypes, particularly in triple-negative disease contexts (Guernet et al., 2016; Domenici et al., 2019; Visvader & Stingl, 2014; Goldhirsch et al., 2013; Padua et al., 2018; Mintz et al., 2020; Mendes de Almeida et al., 2019; Yang et al., 2019; Hannafon et al., 2019; Pulver et al., 2018; Álvarez-Fernández et al., 2017).

4.2. Liver Cancer

In hepatocellular carcinoma, CRISPR-based studies have aided the discovery and validation of candidate targets linked to proliferation, stemness, and therapy resistance. Genome-wide screening and targeted editing have contributed to mechanistic insights and potential biomarker identification in liver tumor biology (Kieckhaefer, Maina, Wells, & Wangensteen, 2019; Liu et al., 2017; Wang et al., 2018a; Han et al., 2015; Wang et al., 2018b; Song et al., 2017; Zhu et al., 2016; Hazafa, Rehman, Jahan, & Jabeen, 2020).

4.3. Lung Cancer

For lung cancer, CRISPR/Cas9 has been applied to explore driver alterations, immune-escape pathways, and resistance mechanisms. Translational work has also intersected

with immunotherapy concepts, including early-phase investigations of edited T-cell strategies, reinforcing clinical feasibility while highlighting the need for stringent safety assessment (Nair, Nair, Veerappan, & Sen, 2020; Lu et al., 2020; Perumal et al., 2019; Eichner et al., 2019; Cheung et al., 2018; Zhang, Lu, Xia, Jiang, & Lv, 2018; Ng et al., 2020).

4.4. Colorectal Cancer

In colorectal cancer, CRISPR-based modeling—especially via engineered organoids and pathway-specific perturbations—has been instrumental in understanding stepwise tumorigenesis and treatment response. These models provide a platform for studying mutation combinations, pathway dependencies, and targetable vulnerabilities in precision medicine frameworks (Kyrochristos & Roukos, 2019; Jubeen et al., 2020; Franko et al., 2016; Matano et al., 2015).

4.5. Prostate Cancer

In prostate cancer, CRISPR/Cas9 has been used to probe androgen receptor signaling, tumor suppressor pathways, and growth-regulatory networks. Editing-based studies have supported both mechanistic clarification and potential target identification relevant to therapeutic development (Blanas et al., 2019; Hsu et al., 2018; Li et al., 2017; Wan et al., 2020; Ferlay et al., 2018; Valcarcel-Jimenez et al., 2019; Fenner, 2020; Batır et al., 2019; Takao et al., 2018; Wei et al., 2018).

5. TECHNICAL AND TRANSLATIONAL CHALLENGES OF CRISPR-Cas9

Before CRISPR/Cas9 can be broadly implemented in routine clinical practice, key challenges must be addressed, including immunogenicity, delivery limitations, off-target

activity, and long-term safety considerations (Kotagama, Jayasinghe, & Abeysinghe, 2019).

5.1. Immunogenicity

Because Cas proteins originate from bacteria, pre-existing adaptive immunity against Cas9 has been reported in humans, including both humoral and cellular responses (Charlesworth et al., 2020). Strategies under investigation include transient expression, ex vivo editing approaches, alternative Cas orthologs, and immunogenicity prediction or mitigation methods.

5.2. Delivery

Safe and efficient delivery remains a major bottleneck. Current approaches include physical (e.g., electroporation), chemical (lipid/polymer nanoparticles), and viral delivery systems, each with distinct advantages and safety constraints (Yip, 2020; Zhang, Shen, Li, & Cheng, 2020; Fajrial, He, Wirusanti, Slansky, & Ding, 2020; Lino, Harper, Carney, & Timlin, 2018; Shah, Aftab, Nisar, Naeem, & Jan, 2021; Xu et al., 2019; Duan et al., 2021; Behr, Zhou, Xu, & Zhang, 2021; Ali, Aslam, Tabasum, & Aslam, 2021). Emerging strategies, including extracellular vesicles, may help overcome limitations related to payload capacity and immunogenicity (Horodecka & Düchler, 2021).

5.3. Off-Target Effects and Genome Integrity

Off-target cleavage and unintended genomic alterations remain central safety concerns. Improved sgRNA design, high-fidelity nucleases, nickase strategies, alternative CRISPR systems (e.g., Cas12a), and anti-CRISPR proteins represent active areas of development to increase precision and controllability (Zhang, Tee, Wang, Huang, & Yang, 2015; Chen, Yao, Zhang, & Fan, 2020; Han, Kah, Pang, & Soh, 2020; Manghwar et al., 2020; Naeem, Majeed, Hoque, & Ahmad, 2020; Collias & Beisel, 2021;

Wang et al., 2021; Paul & Montoya, 2020; Yılmaz, 2021; Morisaka et al., 2019; Liu, Zhang, & Huang, 2020; A & Tanuj, 2020).

Societal acceptance and regulatory readiness also critically shape clinical translation. Somatic editing is generally viewed as more acceptable than germline interventions; however, public concerns related to safety, equity, eugenics, and governance persist, emphasizing the need for transparent regulation and responsible innovation.

6. ETHICAL IMPLICATIONS OF GENE DRIVE IN HUMANS

Human germline genome editing (HHGE), particularly when combined with gene drive technologies, raises distinct and complex ethical challenges that extend beyond those associated with somatic genome editing. A primary concern relates to potential harms arising from technical limitations, including off-target effects, incomplete editing efficiency, mosaicism, and unintended pleiotropic consequences that may manifest across generations (Lander et al., 2019; Dickman, Himmelstein, & Woolhandler, 2017; Nitzbon, Heitzig, & Parlitz, 2017; Hickey et al., 2016; Nestor & Wilson, 2020). Although advances in CRISPR-Cas9 precision and control—demonstrated largely in preclinical and animal gene drive studies—have mitigated some of these risks, the current level of uncertainty precludes definitive conclusions regarding long-term safety in humans. A frequently cited ethical objection to HHGE, especially gene drive–based approaches, is the availability of lower-risk alternatives. Established reproductive and clinical strategies such as preimplantation genetic diagnosis (PGD) combined with in vitro fertilization (IVF), prenatal testing, postnatal medical management, or the use of donor gametes are widely practiced

and avoid permanent alterations to the human germline. From this perspective, the proportionality of risk versus benefit becomes a central ethical criterion. However, it is also recognized that access to these alternatives varies significantly across societies due to economic, cultural, and regulatory factors, complicating blanket ethical judgments. Concerns regarding social justice and inequality constitute a second major ethical dimension (Lander et al., 2019). Critics argue that HHGE could exacerbate existing disparities if access to genome editing technologies is limited to privileged populations, potentially leading to new forms of genetic stratification or discrimination. Conversely, proponents of gene drive–based interventions suggest that, in theory, self-propagating genetic changes could eventually disseminate benefits more broadly without the need for repeated medical interventions. Nevertheless, this speculative advantage must be weighed against unresolved governance, safety, and consent challenges, particularly in the absence of global regulatory consensus. Ethical debates surrounding disability and human diversity further complicate the discourse. Some disability rights advocates most notably within the Deaf community express concern that genome editing aimed at reducing the incidence of certain genetic conditions may reinforce stigmatization or devalue lived experiences associated with disability. While comparable arguments apply to existing practices such as PGD, HHGE raises heightened concerns due to its potential irreversibility and intergenerational reach. Distinguishing between the medical model (focused on disease prevention) and the social model of disability is therefore essential when evaluating the ethical permissibility of germline interventions. Another layer of ethical complexity involves intergenerational relationships and moral responsibility toward future persons. Unlike PGD or non-drive HHGE, gene drive–based HHGE introduces genetic changes that persist automatically across generations, potentially without the knowledge or consent of

future individuals (Rehmann-Sutter, 2018; Puaschunder, 2020; Clarkson, Morrissette, & Regallet, 2001). This self-propagating characteristic challenges foundational bioethical principles, particularly respect for autonomy and informed consent. While some ethical frameworks emphasize a moral obligation to prevent serious disease in future generations, others caution that irreversible genome alterations may impose unjustified constraints on reproductive freedom. Market dynamics and social pressures also warrant ethical scrutiny. The potential for coercion whether driven by commercial interests, societal expectations, or perceived norms of "genetic responsibility" could undermine genuinely autonomous reproductive decision-making (Baylis, 2018; Sah & Fugh-Berman, 2013). Importantly, such pressures are not unique to genome editing; however, the heritable and irreversible nature of gene drive HHGE amplifies their ethical significance.

In summary, ethical objections to HHGE largely apply to both gene drive and non–gene drive genome editing, but gene drive technologies introduce qualitatively distinct concerns related to irreversibility, consent, and intergenerational autonomy. While technical challenges may eventually be addressed, ethical tensions, particularly those involving autonomy, justice, and governance, are unlikely to be resolved through scientific progress alone. Consequently, any consideration of gene drive HHGE must be embedded within robust ethical deliberation, transparent regulatory frameworks, and sustained public engagement to ensure that potential benefits do not come at the expense of fundamental ethical principles.

6.1. Concluding Perspectives

Future developments in CRISPR-based therapeutics will likely prioritize improved specificity, reduced off-target activity, and safer, tissue-appropriate delivery modalities. Base editing,

prime editing, and epigenome editing platforms may expand the therapeutic landscape while minimizing DNA damage and enhancing precision. In parallel, robust regulatory frameworks and ethical oversight must evolve to ensure equitable access, patient safety, and responsible clinical translation. Ultimately, interdisciplinary collaboration among molecular biologists, clinicians, ethicists, and policymakers will be critical for realizing the long-term benefits of genome editing technologies.

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MOLEKÜLER BİYOLOJİ VE GENETİK ALANINDA

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