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İÇİNDEKİLER

Alzheımer Hastalığında Eksozomların Rolü

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1. Alzheimer Hastalığı

Alzheimer Hastalığı (AH), tüm dünyada demansın en yaygın formu olan, kognitif disfonksiyon ve hafıza kaybı, motor fonksiyon bozuklukları ile karakterize, ilerleyici özellikte nörodejeneratif bir hastalıktır. Hastalığın henüz ilerleyişini durdurabilecek bir tedavi yönteminin olmaması, var olan tedavilerin semptomatik olması sebebiyle AH araştırmaları günümüzde oldukça önemli bir araştırma konusu olma özelliğini sürdürmektedir. AH hem hasta yakınlarına hem de sağlık sistemine ciddi psikolojik ve ekonomik yük oluşturmaktadır. Dünya Sağlık Örgütü'nün Mart 2025 tarihli raporunda 2021 yılı itibariyle dünya genelinde 57 milyon demanslı hastanın yaşadığı, her yıl bu sayıysa 10 milyon yeni vakanın eklendiği ve bu hastaların yaklaşık %60-70'inin ise Alzheimer hastası olduğu belirtilmiştir (Dünya Sağlık Örgütü, 2021).

Hastalığın en önemli belirtileri arasında ise yaşanan olayları unutmak, konuşma güçlüğü, muhakeme zorluğu, zaman algısında sorunlar, günlük aktiviteleri sürdürmede zorluk sayılabilir (Arshavsky, 2020). Hastalığın temel hücresel belirtileri interselüler alanda senil amiloid beta plak birikimi, intraselüler alanda hiperfosforile taudan oluşan nörofibriler yumaklardır. Ayrıca sinaps fonksiyon bozukluğu, oksidatif strese bağlı hasar, nöroinflamasyonda artma, sinaps ve nöron kaybı, mitokondriyal hasar ve beyin atrofisi de gözlemlenmektedir (Jellinger, 2021; Monteiro vd., 2023).

Hastalığın en önemli risk faktörleri yaşlanma, hipertansiyon, hiperglisemi, obezite, aşırı sigara ve alkol kullanımı, fiziksel aktivitede azlık ve düşük eğitim seviyesidir (Abeysinghe vd., 2020; Nasb vd., 2023).

Alzheimer hastalığının ailesel (erken başlangıçlı, early onset) ve sporadik (geç başlangıçlı, late onset) olmak üzere iki türü bulunmaktadır. Ailesel AH'de amiloid beta üretimini artıran APP, PSEN1 ve PSEN2 mutasyonları gözlemlenmektedir. Sporadik AH ise daha kompleks ve multifaktöriyel bir hastalıktır (Roda vd., 2022; Sims vd., 2020).

Hastalık patolojisini açıklamak için birçok hipotez öne sürülmüştür. Bu hipotezlerden en fazla çalışılanı amiloid kaskad hipotezine göre amiloid beta proteini AH'de nörodejenerasyonu başlatan ve ardından taunun fosforilasyonu, oksidatif stres, inflamasyon gibi süreçleri tetikleyen ana faktördür (Roda vd., 2022). Tau hipotezine göre ise, tau fosforilasyonu amiloid beta plak oluşumunu takip eden bir süreç değil hastalığı başlatan süreçlerdendir. Hiperfosforile tau proteinin beyindeki yayılımı ile kognitif fonksiyonlardaki bozulma ve hafıza problemleri paralel ilerlemektedir (Basheer vd., 2023; García-Morales vd., 2021). Kolinerjik hipoteze göre ise hastalığa yol açan patolojilerden birisi kolinerjik

nöronlardaki kayıp sebebiyle hafıza ve kognitif fonksiyonlarda rol alan asetilkolin nörotransmiterinin üretimindeki azalmadır (Nasb vd., 2023).

Senil amiloid beta plakları ve nörofibriler yumaklar, nöroinflamasyon, sinaps kaybı ve oksidatif stresi tetiklemektedir. Nöroinflamasyon AH'de ortaya en erken çıkan sonuçlardan birisidir, toksik amiloid beta ve tau IL-6, TNF-a ve IL-1B gibi sitokinlerin salgılanmasını uyararak mikrogliaları aktifleştirerek kronik inflamasyona ve ardından nörotoksisiteye sebep olur (Arshavsky, 2020; Wong-guerra vd., 2023). Sinaps kaybı AH'deki kognitif bozulmanın en önemli sebeplerindendir. Amiloid beta NMDR reseptörü, tau ise muskarinik reseptörler üzerinden sinaptotoksisiteye sebep olur (Tzioras vd., 2023). Oksidatif stres durumunda reaktif oksijen ve reaktif nitrojen türlerinin birikimi gözlemlenir. Oksidatif stres mitokondriyal fonksiyonlarda bozulmaya, inflamasyona ve apoptoza yol açmaktadır (Monteiro et al., 2023).

2. Eksozomlar

Ekstraselüler veziküller, eksozom, mikrovezikül ve apoptotik cisimlerden oluşan farklı büyüklük ve yapıda veziküllerdir. Mikroveziküller 100-1000 nm çapında hücre membranından direkt tomurcuklanma ile oluşan veziküllerdir. Apoptotik cisimler 100-2000 nm çapında apoptotik hücrelerin membranlarından üretilen veziküllerdir. Eksozomlar lipit yapıda membrandan oluşan, hücre membranından köken alan nano boyutta veziküllerdir. Çapları 30-150 nm arasında değişen eksozomlar, endozomal kökenli biyonanopartiküllerdir (Jan vd., 2021; Sun & Chen, 2024).

Eksozomlar; sfingomyelin, kolesterol gibi membran lipitleri, CD9, CD81, Alix, Tsg101 gibi proteinler, siRNA, miRNA, lncRNA gibi kodlamaya yapmayan RNAlar, mRNA gibi makromolekülleri taşımaktadır. Taşıdıkları lipit, protein ve nükleik asit kargoları sebebiyle hücre-hücre iletişiminde görev almaktadırlar. Eksozomların köken aldıkları hücrelere göre içerdikleri moleküller değişmektedir ve oldukça heterojen bir yapı sergilemektedirler. Kan, serum, plazma, anne sütü, idrar, sinovial sıvı, beyin omurilik sıvısı, gözyaşı, tükürük gibi tüm vücut sıvılarında bulunabilmektedirler (Huang vd., 2023; Mohamed vd., 2025).

Keşfedildikleri 1980'li yıllarda eksozomların sadece hücre içi istenmeyen moleküllerin atılmasında görevli olduğu düşünülürken, ilerleyen yıllarda taşıdıkları nükleik asitlerle hedef hücrede ekspresyon değişikliğini tetikleyebildiği, içerdiği biyomoleküller ile hücre sinyalizasyonunda görevli olduğu anlaşılmıştır. Patolojik koşullarda kargo içeriği değişen eksozomların

hastalık patolojilerinin yayılmasında görevli olduğu da düşünülmektedir (Gurunathan vd., 2019).

2.1. Eksozom Biyogenezi

Eksozom biyogenezinde ilk adım hücre membranının hücre içine doğru tomurcuklanarak erken endozomları oluşturmasıdır. Ardından endozom membranı invajinasyon yaparak multiveziküler cisimciklerin içinde yer alan intraluminal vezikülleri oluşturur. Multiveziküler cisimcikler ya lizozomla birleşir ya da hücre membranıyla füzyon yaparak içerisindeki intraluminal vezikülleri eksozom olarak hücre dışı alana bırakır (Satao & Doshi, 2025).

ESCRT (Endosomal Sorting Complex required for Transport) proteinleri ESCRT-0, ESCRT-I, ESCRT-II, ESCRT-III eksozom olgunlaşması, tomurcuklanması, intraluminal veizküllerin ayrılması ve kesilmesi gibi süreçlerde yer alır. RAL- 1 GTPaz ve RAB proteinleri mutiveziküler cisimciklerin membran ile füzyon yapmasında görevlidir. Tetraspaninler, sfingomyelin ve seramid de eksozom biyogenezinin birincil mediatörleridir (Hyenne vd., 2015; Satao & Doshi, 2025).

Eksozomlar hedef hücreye makropinositoz, fagositoz, reseptör aracılı endositoz, lipit salı (raft) oluşumu ve membran füzyonu ile alınabilir. Ayrıca hücre yüzeyindeki reseptörler ile etkileşime girerek hücre içine alınmadan da çeşitli hücre sinyalizasyon yolaklarını tetikleyebilmektedir (Van Niel vd., 2018).

3. Alzheimer Patolojisinde Eksozomlar

Kan beyin bariyerini geçme yeteneğine sahip olan eksozomlar, bu özellikleri sayesinde periferal maddeleri merkezi sinir sistemine (MSS) taşıyarak ve MSS'deki hücreler arasında iletişimi sağlayarak hücre sinyalizasyonunda görev alır. Eksozomlar taşıdıkları nörotransmiter, nöromodulatör ve sinaptik proteinler ile MSS'de, nörogenez, sinaptik aktivite, sinaptik plastisite, miyelinasyon ve nöronal rejenerasyonda görev alır (Bir vd., 2024; Liu vd., 2024). MSS'de eksozom üretimi GABA antagonistleriyle artarken, NMDA ve AMPA antagonistleri ile eksozom üretimi baskılanmaktadır. Eksozomlar nörodejeneratif hastalıklarda toksik proteinleri nöronlardan glialara ya da nöronlardan nöronlara taşıyabilmektedir (Ashique vd., 2024).

Amiloid prekürsör proteini (APP), APP metabolitleri ve APP işlenmesinde görevli enzimler nöronal hücre kültürlerinde eksozomlar aracılığıyla ekstraselüler alana salınmaktadır (Satao & Doshi, 2025). Aynı şekilde amiloid beta 1-42 proteinin de multiveziküler cisimlerde birikmeye başladığı belirlenmiştir (Ashique vd., 2024). Amiloid beta üretiminde görevli enzimlerden

BACE1, Presenilin1 ve Presenilin2'nin de eksozomlara paketlendiği bildirilmiştir (Bir vd., 2024). Ayrıca post-mortem hasta doku örneklerinde amiloid beta plaklarında eksozom belirteçlerinden olan Alix proteinine rastlanmıştır (Rajendran vd., 2006).

Eksozomlarda kontrol grubuna kıyasla daha fazla oligomerik taunun bulunduğu, eksozomlardaki fosforile taunun hedef hücrelerde tau agregasyonuna sebep olduğu, böylece AH'de tau patolojisini yaygınlaştırdığı tespit edilmiştir (Polanca vd., 2016).

Alzheimer hastalarının kanlarından elde edilen eksozomlarda APP ekspresyonunu baskılayan miRNA'lardan miR-20a-5p'nin ekspresyonu azalırken, bir başka çalışmada hastaların kan ve beyin omurilik sıvısından elde edilen ekzosomlarda miR-193b'nin ekspresyonunun azaldığı gösterilmiştir (Liu vd., 2014; Wang vd., 2022).

Aynı zamana sinaptik proteinler olan sinaptofizin, sinaptotagmin, sinaptopodin, nörogranin gibi proteinlerin Alzheimer hastalarında plazmadan izole edilen nöronal kökenli eksozomlarda miktarlarının azaldığı belirlenmiştir (Liu vd., 2024).

Bununla birlikte eksozomlar AH'de nöroinflamasyon sürecine de katkı sunmaktadır. Astrosit kökenli eksozomların C1q, C4b, ve faktör-B gibi komplement proteinlerini ve IL-1, TNF-α, ve IL-1β gibi proinflamatuar belirteçleri sağlıklı kontrollere kıyasla daha fazla taşıdığı belirlenmiştir (Goetzl vd., 2018).

Mikroglialarn çözebilir amiloid betayı internealize edip parçalamasının yanında çözünebilir amiloid betaları içeren eksozomları üretip ekstraselüler alanda amiloid beta agregasyonuna katkıda bulunduğu gösterilmiştir (Ashique vd., 2024).

Oksidatif stres durumunda eksozomların salınımı artmakta ve içerikleri değişmektedir. Bu eksozomlarda hücre sağkalımı ile ilgili genlerin ekspresyonu azalırken apoptoz ile ilişkili genlerin ekspresyonu artmaktadır. Ayrıca eksozomlarda yer alan lipit moleküllerinin de oksidasyonu ile, eksozomlar hedef hücrelerinde inflamatuar yanıtı artırmaktadır. Bununla birlikte, artan oksidatif stres eksozom üretimini de artırmaktadır (Chiaradia vd., 2021; Manček-Keber vd., 2015; Yang vd., 2019).

Tüm bu sonuçlarla birlikte eksozomların Alzheimer hastalığının yayılmasına ve ilerlemesine mi yoksa endositik yollar ile toksik proteinleri temizleme sürecine katkı mı sağladığı hâlen tartışma konusudur (Huber & Wang, 2024). Eksozomlar

taşıdıkları sistatin C, neprilisin, insulin degrade eden enzim (IDE), endotelin dönüştürücü enzim (ECE- endothelin converting enzyme) gibi proteinler ile amiloid beta degradasyonunu sağlayarak AH'de protektif rol oynayabilirler. (Bulloj vd., 2010; Pacheco-Quinto vd., 2019; Sun & Chen, 2024)

4. Tanısal Biyobelirteç Olarak Eksozomlar

Alzheimer hastalığı özelinde biyobelirteçler, amiloid beta birikimi (A), patolojik tau (T) ve nörodejenerasyon (N) ile ilişkili olmasına göre sınıflandırılmıştır. ANT sınıflandırması görüntüleme yöntemleri ve vücut sıvıları da dahil olmak üzere birçok biyobelirteci patolojik süreçlerle ilintisine göre sınıflamaktadır. Halihazırdaki yöntemler, beyindeki amiloid beta plak ölçümü için PET taramaları, beyin omurilik sıvısında tau analizi gibi yöntemleri içermektedir. Bu yöntemler AH'nin erken evrelerini tespitte yetersiz olmakla birlikte invaziv, pahalı ve zor ulaşılabilen yöntemlerdir (Bir vd., 2024; Chapleau vd., 2022).

Hastalık durumlarında değişen içerikleri ve tüm vücut sıvılarında bulunmaları sayesinde, eksozomlar güçlü biyobelirteç adayları olarak öne çıkmaktadır. Nörodejeneratif hastalıklar özelinde ise kan beyin bariyerini geçmeleri sayesinde eksozomlar MSS'den periferal dokulara, periferal dokulardan MSS'ye eksozomal biyobelirteçlerin geçişi anlamına gelmektedir (Liu vd., 2024). Eksozomlar hem likit biyopsi materyalleri olarak hastalık teşhisinin konulmasında; hem de hastalık oluşumundan önce prediktif olarak ve hastalığın ilerleyişinin/evresinin takip edilebileceği biyobelirteçler olarak kullanılabilme potansiyelindedir (Li vd., 2024).

Eksosomal AB, total ve fosforile tau ölçümü, beyin omurilik sıvısı ölçümleri gibi, kognitif fonksiyonlarda bozulmayı gösterebilmektedir (Liu vd., 2024). Eksozomal amiloid beta ölçümlerinin, dolaşımdaki serbest amiloid beta ölçümlerine kıyasla amiloid beta plakları ve kognitif bozulma ile daha güçlü bir korelasyon gösterdiği belirlenmiştir (Lim vd., 2019). Beyin kökenli eksozomlardaki fosforile tau (p-S181-tau ve p-S396-tau) seviyelerinin ölçümü de hastaları kontrol grubundan ve hafif bilişsel bozukluktan (MCI-mild cognitive impairment) ayırmada etkili olmakta, hastalık başlamadan on yıl önce bu proteinlerin eksozomdaki ekspresyonları artmakta ve erken teşhise olanak sağlamaktadır (Fiandaca vd., 2015; Jia vd., 2019).

miRNAların biyobelirteç olarak hedeflendiği çalışmalarda ise hasta kanlarından ya da beyin omurilik sıvısından elde edilen eksozomlarda miRNA'ların ekspresyon düzeyleri incelenmektedir. Örneğin Alzheimer hastalarının BOS kökenli eksozomlarında miR-9 ve miR-598 ekspresyonun

yükseldiği belirlenmiştir. Bu miRNA'ların eksozomdaki miktarı BOS'taki serbest formlarına göre çok daha yüksek olduğundan bu durum spesifik bir eksozomal enkapsülasyonunu göstermekte ve eksozomal miRNA'ları önemli biyobelirteç adayları olarak öne çıkarmaktadır (Riancho vd., 2017). Bir başka çalışmada eksozomal miR-16-5p'nın erken başlangıçlı AH'de ekspresyonunda disregülasyonu belirlenmiş ancak geç başlangıçlı AH'de bir fark gözlemlenmemiştir, bu durum sonucunda eksozomal miR-16-5p erken başlangıçlı AH için ayrıcı bir biyobelirteç adayı olarak öne çıkmıştır (McKeever vd., 2018).

5. Eksozom Tabanlı Tedavi Yaklaşımları

Biyolojik nanopartikül yapısındaki eksozomlar, kan beyin bariyerini geçebilmeleri, immün yanıt oluşturmamaları ve taşıdıkları kargo moleküllerini hedef hücreye taşıyabilme kapasitelerinden dolayı tedavi süreçlerinde ilaç taşıma sistemleri olarak da değerlendirilmeye başlanmıştır. Eksozomlar intranazal, intravenöz, oral vb. olarak verilebilen birçok ilaç formülasyonu hazırlamaya uygun yapıdadır (Chunhui vd., 2025).

Mezenkimal kök hücreler tarafından üretilen eksozomların AH modellerinde kullanılmasıyla hafıza ve bilişsel fonksiyonlarda iyileşme; amiloid beta plak oluşumunda ve nöroinflamasyonda azalma gözlemlenmiştir (Ding vd., 2018).

İlaç taşıma sistemi olarak eksozomların kullanıldığı çalışmalarda ise, eksozomlara inkübasyon, elektroporasyon, sonikasyon, membran geçirgenliğini artıran kimyasallar ile muamele, ekstrüzyon ve klik kimyası gibi çeşitli yöntemler ile eksozom izolasyonu sonrası ve inkübasyon ve transfeksiyon gibi yöntemlerle eksozom izolasyonu öncesi ilaç yüklemesi yapılabilmektedir. Eksozomlara doğal bileşikler, küçük moleküller, ilaçlar, kodlama yapmayan RNA'lar gibi birçok molekül terapötik amaçlı yüklenebilmektedir (Vader vd., 2016). AH'de çeşitli çalışmalarda BACE1'i hedefleyen siRNA, kurkumin, kuersetin, neferin gibi moleküller yüklenerek nöroprotektif etki, nöroinflamasyonda azalma, kognitif fonksiyonlarda iyileşme, amiloid beta yükünde azalma gözlemlenmiştir (Alvarez-Erviti vd., 2011; Qi vd., 2020; Tang vd., 2022; Wang vd., 2019).

6. Sonuç

Alzheimer hastalığı, multifaktöriyel karakterde karmaşık nörodejeneratif bir hastalık olarak halihazırda tüm dünyada önemli bir halk sağlığı sorunu olmaya devam etmekte, bu sebeple tanı ve tedavi geliştirme çalışmaları çok boyutlu bir yaklaşım gerektirmektedir.

Son yıllarda eksozomlar yapı ve fonksiyonları sebebiyle dikkat çeken biyolojik yapılar olarak öne çıkmaktadır. Alzheimer hastalığı özelinde eksozomların hem patolojik süreçlerin yayılımında rol alabileceği hem de toksik proteinleri uzaklaştırıcı protektif mekanizmalarla hastalığın progresyonunu yavaşlatabileceği yönünde bulgular mevcuttur. Ayrıca, taşıdıkları özgül biyomoleküller sayesinde eksozomlar, tanı süreçlerinde likit biyopsi temelli non-invaziv biyobelirteç adayları olarak da değerlendirilmektedir. Aynı zamanda ilaç taşıma sistemleri olarak da tedavi yaklaşımlarında klinik araştırma çalışmalarında sıklıkla kullanılmaktadır.

Bu bulgular doğrultusunda, Alzheimer hastalığı patolojisinin anlaşılması, erken tanısı ve hedefe yönelik tedavi geliştirilmesi süreçlerinde eksozom temelli yaklaşımlar gelecek vaat etmektedir.

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Stem Cell and Genetıc Science: Biological Basis, Molecular Technologies and Clinical Applications

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Introduction

Stem cell science stands at the forefront of modern biomedical research, offering unparalleled promise for the treatment of a wide spectrum of degenerative, autoimmune, and genetic disorders (Zakrzewski, Dobrzynski, Szymonowicz, & Rybak, 2019). Stem cells are characterized by their unique ability to self-renew and differentiate into specialized cell types, positioning them as a cornerstone of regenerative medicine (Hoang et al., 2022). Their discovery and therapeutic potential have evolved remarkably, from historical milestones like the isolation of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) to the development of cell-based therapies for conditions once deemed incurable (Hussen et al., 2024).

While stem cell therapy holds transformative clinical potential, its practical application remains constrained by several factors, including immunogenicity, tumorigenesis, and ethical controversies surrounding the use of embryonic tissues (Volarevic et al., 2018). In this context, stem cell-derived exosomes (SC-Exos) have emerged as a safer, cell-free alternative that retains the therapeutic efficacy of their parent cells without associated safety and ethical concerns. SC-Exos demonstrate potent effects in immunomodulation, tissue regeneration, and anti-inflammation, making them ideal candidates for diverse surgical and non-surgical applications (Tan et al., 2024).

Together, the progress in stem cell and exosome research represents a paradigm shift in regenerative medicine. By harnessing both cellular and extracellular strategies, current approaches are paving the way toward personalized, effective, and ethically responsible therapeutic interventions across various medical disciplines (Seth et al., 2025).

The primary aim of this chapter is to integrate the fundamental principles of stem cell biology and genetic science, and to comprehensively present the innovative molecular approaches and clinical applications emerging from the intersection of these two fields. The chapter will elaborate on the biological classification of stem cells, mechanisms of genetic differentiation, gene editing technologies (e.g., CRISPR/Cas9), disease modeling, and translational approaches to therapy. Additionally, it aims to address ethical and societal dimensions, thereby contributing a scientific perspective to current debates in the field.

In this context, the chapter seeks to achieve the following objectives:

- To define the types of stem cells and their genetic potentials,
- To explain genetic and epigenetic regulation in cellular differentiation processes,

- To present the impact of modern genetic engineering techniques on stem cells,
- To evaluate genetic disease models and stem cell-based therapeutic approaches through examples,
- To analyze the integration of stem cell and genetic technologies in clinical applications,
- To raise awareness regarding ethical, legal, and social considerations.

The Intersection of Stem Cells and Genetics

Stem cells are undifferentiated cells with the ability to transform into various specialized cell types. Genetic science, on the other hand, investigates how hereditary information within cells influences the development, function, and diseases of an organism. The integration of these two fields lays the foundation for innovative applications such as disease modeling, gene therapy, and tissue engineering (Weissman, 2002). IPSCs have revolutionized regenerative medicine with their ability to proliferate indefinitely and differentiate into nearly any cell type. They offer a human-based platform to model development, study diseases, and screen for new drugs in vitro. Advances in reprogramming and differentiation protocols have made iPSC technology increasingly accessible and clinically relevant. Furthermore, genetic modifications to iPSCs enable both disease modeling and the design of targeted gene therapies (Cerneckis, Cai, & Shi, 2024).

iPSC-based disease models allow for functional genomic screenings using CRISPR to uncover gene-disease relationships. These technologies facilitate unbiased identification of disease modifiers and therapeutic targets at high throughput. CRISPR-interference and activation screens in PSC-derived models offer insight into gain- and loss-of-function effects. Despite technical challenges, such platforms are crucial for advancing drug discovery (Shevade, Peddada, Mader, & Przybyla, 2023). Mesenchymal stem cells (MSCs) represent a potent tool that bridges cellular and genetic therapies, offering differentiation capacity alongside immunomodulatory properties. Genetically modified MSCs can be engineered to deliver therapeutic agents directly to disease sites. Their ability to home to damaged tissues and secrete bioactive molecules underlines their promise in clinical gene therapy. However, safety and targeting issues remain critical challenges (Myers et al., 2010).

The single-cell eQTLGen consortium integrates genetic variation with cell-type specific transcriptomic data to clarify the genetic architecture of complex diseases. Single-cell technologies allow mapping of gene regulatory networks and identification of cell-specific disease mechanisms. This level of resolution can inform personalized therapeutic strategies. It marks a shift from bulk analyses

to precise, cell-resolved interpretations of genetic influence (Van der Wijst et al., 2019).

Stem Cell Types and Genetic Characteristics

Stem cells are classified as totipotent, pluripotent, and multipotent. Totipotent cells are derived from the zygote, whereas pluripotent cells represent embryonic stem cells. Multipotent stem cells found in adult tissues possess a limited capacity for differentiation. IPSCs are generated by genetically reprogramming somatic cells (Takahashi & Yamanaka, 2006). Human pluripotent stem cells (hPSCs), while offering immense therapeutic potential, tend to acquire genetic variations during extended culture. These culture-induced aberrations include chromosomal gains, sub-karyotypic mutations, and single nucleotide variants that may compromise the safety and efficacy of stem cell-based applications. Such mutations can confer selective growth advantages, raising concerns about tumorigenicity and reproducibility. Thus, monitoring and minimizing genetic instability remains crucial for clinical translation of hPSC-derived therapies (Vales & Barbaric, 2024).

Pluripotent stem cell (PSC) repositories predominantly derive from individuals of European ancestry, limiting the genetic scope of disease modeling. Including genetically diverse iPSC lines from underrepresented populations enhances discovery of ancestry-specific risk variants. Such diversity enables more equitable and precise biological insights, particularly in drug responses and disease susceptibility. Expanding genetic representation in PSC models is therefore critical for personalized regenerative medicine (Ghosh, Nehme, & Barrett, 2022). HOX genes, although silenced in early pluripotent stem cells, play key roles in defining cellular identity and directing lineage-specific differentiation. In adult stem cells such as mesenchymal and hematopoietic stem cells, HOX expression reflects tissue origin and developmental history. These genes act as master regulators of spatial identity through intricate signaling interactions including Wnt, TGF-β, and retinoic acid pathways. Understanding HOX regulation can enhance targeted differentiation in regenerative applications (Steens & Klein, 2022). Embryonic stem cells (ESCs), derived from the inner cell mass of blastocysts, possess the potential to generate all three germ layers. Recent advancements allow the generation of synthetic embryo models from ESCs, recreating early mammalian development in vitro. These models facilitate investigation of lineage specification and early morphogenesis in a scalable, ethically viable manner. ESC-based systems thus provide powerful platforms for studying development and modeling genetic diseases (Kim, Kim, & Shin, 2023).

Stem Cell Differentiation and Gene Regulation

Stem cell differentiation is accompanied by dramatic and coordinated changes in gene expression that are maintained over time through epigenetic regulation. These include DNA methylation, histone modification, and non-coding RNA-mediated silencing and activation. Such modifications ensure the stable silencing of self-renewal genes and activation of lineage-specific programs. Epigenetic memory, established during development, plays a crucial role in sustaining differentiated states(Wu & Sun, 2006). In the intestinal epithelium, stem cell differentiation is tightly regulated by enhancer activity responsive to niche-derived cues. These enhancers integrate Wnt, Notch, and other signals to promote precise transcriptional outputs in daughter cells. Chromatin accessibility and histone acetylation patterns dynamically shift as stem cells commit to absorptive or secretory lineages. This plasticity ensures robust yet flexible lineage commitment essential for gut homeostasis (Verzi & Shivdasani, 2020).

Gene expression profiling in stem cells reveals unique transcriptional signatures distinguishing them from progenitor or fully differentiated cells. Key pluripotency genes such as Oct4, Sox2, and Nanog are tightly regulated at both transcriptional and epigenetic levels. The transition to differentiation involves downregulation of these core genes and induction of lineage-specific regulators. These findings underscore the utility of transcriptomic analyses in defining stem cell identity (Liang, Russell, Walworth, & Chen, 2009). A multi-omics approach combining mRNA, protein, and microRNA data reveals the layered complexity of gene regulation during stem cell differentiation. Time-resolved analyses show that transcriptional changes precede and predict later protein expression dynamics. MicroRNAs act as modulators of translational efficiency, refining gene expression outputs beyond transcription. These insights inform dynamic models of cell fate transitions and regenerative engineering (van den Berg, Berenger-Currias, Budnik, Slavov, & Semrau, 2023).

Genetic Engineering Methods and Their Use in Stem Cells

Human-induced pluripotent stem cells (hiPSCs) offer a powerful model for studying human diseases and pharmacological responses due to their ability to mimic tissue-specific physiology and pathology. The CRISPR/Cas9 system enhances this utility by enabling precise gene editing within hiPSCs, allowing for correction of disease-causing mutations. These cells can be derived from minimally invasive samples such as urine, blood, or skin biopsies, facilitating accessibility. When cultured in three-dimensional systems like organoids, they closely replicate native tissue architecture and microenvironments, improving clinical relevance (De Masi, Spitalieri, Murdocca, Novelli, & Sangiuolo, 2020). Hematopoietic stem/progenitor cell (HSPC) gene therapy involves genetically modifying harvested HSPCs to restore or replace defective functions upon

transplantation. The use of viral vectors as vehicles for gene delivery has significantly contributed to its clinical success, particularly for monogenic diseases. New technologies like site-specific gene editing are expanding the scope of treatable conditions and improving safety. Advances in the biological characterization of HSPCs are crucial for designing the next generation of transformative therapies (Ferrari et al., 2023).

Genetic engineering of stem cells prior to transplantation improves their survival and function within the host environment. These modified cells can be protected against immune rejection, oxidative stress, and apoptosis, thereby enhancing therapeutic outcomes. Transgenes are often delivered using modular vectors tailored for specific clinical goals. The metaphor of a 'Trojan horse' captures this approach—genetically enhanced cells infiltrate the host and deliver therapeutic payloads where needed (Phillips & Tang, 2008). CRISPR/Cas9 technology has emerged as an indispensable tool in stem cell research focused on degenerative diseases. Its application in mesenchymal stem cells and other lineages allows for precise correction of point mutations and modeling of disease mechanisms. When coupled with patient-derived stem cells, CRISPR enables truly personalized approaches to gene therapy. Ongoing developments continue to refine delivery systems and reduce off-target effects, enhancing both safety and efficacy (Valenti, Serena, Carbonare, & Zipeto, 2019).

Stem Cell and Genetics Based Disease Models

Human-induced pluripotent stem cells (hiPSCs) serve as a valuable platform for modeling genetic diseases, owing to their patient-specific origin and pluripotent capabilities. CRISPR/Cas9 technology enables precise gene editing in these cells, allowing for targeted correction of disease-associated mutations. When integrated into three-dimensional culture systems like organoids, edited hiPSCs can mimic tissue-specific pathophysiology with high fidelity (Figure 1). These combined tools offer unprecedented potential in drug screening and personalized therapeutic development (De Masi et al., 2020).

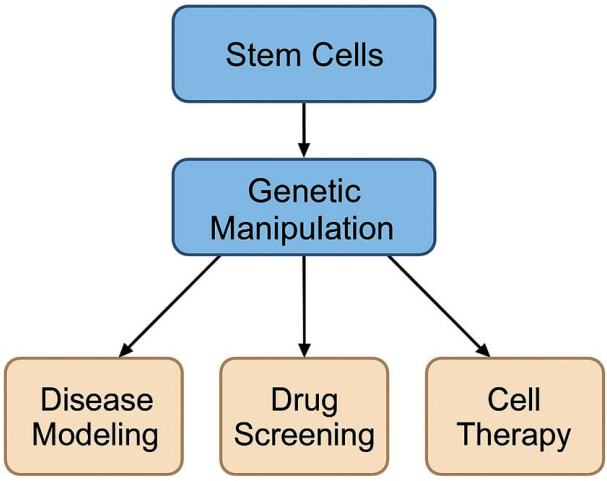

Figure 1. Schematic representation of CRISPR/Cas9 application in human-induced pluripotent stem cells (hiPSCs). This diagram illustrates the workflow from donor cell collection and reprogramming to gene editing, organoid formation, and application in disease modeling and drug screening (De Masi et al., 2020).

The value of genome-edited pluripotent stem cells lies in their ability to model monogenic disorders such as MODY or neonatal diabetes in a controlled and scalable manner. Beyond simply modeling disease, these cells enable the discovery of disease modifiers through comparative genomic studies and isogenic line generation. They are especially useful in recapitulating β-cell dysfunction, a hallmark of many diabetes subtypes, thereby enabling the study of gene-function relationships at the cellular level. Such platforms facilitate not only functional annotation of variants but also personalized drug response prediction (George, Leavens, & Gadue, 2021).

Marfan syndrome represents a quintessential case where human pluripotent stem cells have enabled modeling of complex genotype-phenotype relationships. The availability of over 50 hPSC lines has allowed researchers to model cardiovascular and skeletal abnormalities in vitro with remarkable fidelity. These patient-specific models also provide an opportunity to study how different FBN1 variants lead to variable clinical manifestations. Such systems will likely pave the way for risk stratification and tailored treatment strategies in Marfan patients (Aalders, Muino Mosquera, & van Hengel, 2024).

Stem cell models have also transformed our understanding of neurodegenerative and cardiac diseases through iPSC-based systems. For example, diseases like SMA and Huntington's disease have been effectively modeled, revealing cellular phenotypes and response to candidate drugs. This is possible due to iPSCs' ability to differentiate into neurons or cardiomyocytes,

allowing detailed analysis of disease progression in human cellular context. Furthermore, gene editing in these models supports the identification of biomarkers and drug screening targets (Table 1) (Sterneckert, Reinhardt, & Scholer, 2014).

Table 1. Selected Examples of Disease Models Using Human iPSCs (Sterneckert et al., 2014)

Disease	**Mutant Gene**	**Cell Type Modeled**	**Application**
A1AT Deficiency	A1AT	Hepatocytes	Gene correction
Wilson's Disease	ATP7B	Hepatocytes	Phenotypic rescue
Long QT Syndrome	KCNQ1 / KCNH2	Cardiomyocytes	Drug screening
Huntington's Disease	HTT	Neurons	Phenotype modeling
Spinal Muscular Atrophy	SMN1	Motor Neurons	Therapeutic screening

Stem cell-based models are now integral to both disease modeling and regenerative therapies, offering insights into developmental biology and therapeutic efficacy. This is especially relevant in organoid systems where disease mechanisms can be studied in three-dimensional cultures. The combination of CRISPR/Cas9 and stem cell technology creates a potent toolset for both basic research and translational medicine. Such systems are also scalable and adaptable for high-throughput drug screening (Bai, 2020).

Clinical Applications: Cellular and Genetic Therapeutic Approaches

Gene therapy has revolutionized the management of rare monogenic diseases by allowing correction or compensation of defective genes directly within patient cells. Techniques range from gene addition and suppression to genome editing, including CRISPR-based approaches recently approved for conditions like β-thalassemia and sickle cell disease. Ex vivo and in vivo gene transfer strategies are now routinely employed in clinical settings for disorders such as spinal muscular atrophy and inherited retinal dystrophies. While safety and regulatory challenges remain, the therapeutic benefits and transformative potential of these interventions are clear (Ay & Reinisch, 2025).

The clinical landscape has also seen a remarkable rise in hematopoietic stem cell gene therapy (HSCGT), particularly in treating inherited blood disorders. Conditions like ADA-SCID, Wiskott-Aldrich syndrome, and β-thalassemia have responded successfully to gene addition via lentiviral vectors or CRISPR-mediated editing, offering patients long-term or curative outcomes. Advances in gene editing technologies, including base and prime editing, are further refining the precision and efficacy of HSCGT. However, long-term follow-up and refined delivery systems are necessary to fully ensure therapeutic durability and safety (Kohn, Chen, & Spencer, 2023).

Meanwhile, cell therapies—especially mesenchymal and hematopoietic stem cells—are being harnessed for regenerative, immunological, and oncological applications. The FDA has approved several products (e.g., KYMRIAH™, PROVENGE®) based on either autologous or allogeneic cellular immunotherapies, demonstrating strong clinical utility in various cancers and immune conditions. Notably, multicellular therapies are gaining momentum due to their synergistic effects and improved clinical outcomes compared to unicellular approaches. Regulatory frameworks are evolving in parallel to support their broader adoption (El-Kadiry, Rafei, & Shammaa, 2021).

A distinct but complementary path in gene therapy involves treatments for rare diseases, where traditional drugs often fail due to economic or biological limitations. AAV-based vectors (e.g., Luxturna®), antisense oligonucleotides (e.g., Spinraza®), and CAR-T cell therapies (e.g., Yescarta®) have entered clinical use, showing efficacy against otherwise intractable genetic and malignant disorders. These platforms not only restore protein function but also offer scalable, customizable solutions for future rare disease management. As technology advances, combination approaches involving genome editing and RNA-based modulation are poised to further enhance clinical outcomes (Papaioannou, Owen, & Yanez-Munoz, 2023).

Ethical, Legal and Social Aspects

The use of embryos in stem cell research, germline editing, and the privacy of genetic data are central issues in ethical debates. The ISSCR and CIOMS guidelines provide important frameworks in navigating these concerns (Lo & Parham, 2009). The rapid advancement of CRISPR-Cas9 has amplified ethical concerns, particularly regarding its application to the human germline, which may introduce heritable changes without a clear understanding of long-term consequences. Discussions emphasize the need for robust international regulation that includes perspectives from not only scientists but also ethicists and policymakers to prevent misuse and ensure equitable benefit. Bioethics, therefore, becomes a necessary framework to guide the responsible development and application of gene editing technologies. These reflections underscore the

importance of establishing limits based on moral principles and societal values (Ozturk Turkmen & Arda, 2008).

In Turkey, legal and ethical frameworks for stem cell research have been evolving, but challenges remain due to inconsistencies in regulation and limited public awareness of patient rights. The moral legitimacy of using embryos, especially surplus embryos from IVF, has been a topic of intense debate, reflecting wider societal values and religious perspectives. Informed consent, transparency in donor use, and protection against the commodification of biological material are emphasized as core ethical concerns. These issues demonstrate the necessity for national and international standards to safeguard both individual rights and scientific integrity (Ozturk Turkmen & Arda, 2008).

Genome editing technologies like CRISPR-Cas9, while offering unprecedented therapeutic potential, also raise bioethical dilemmas related to safety, informed consent, and the potential for eugenic practices. The creation of genetically modified embryos—even with the intent to cure disease—may lead to unforeseen consequences and societal inequality. Thus, global legislation is called for to govern the responsible use of genome editing in medicine, agriculture, and beyond. Regulatory policies must balance scientific freedom with ethical boundaries to ensure public trust and safety (Ayanoglu, Elcin, & Elcin, 2020).

The ISSCR 2021 Guidelines provide updated frameworks for stem cell and embryo research, categorizing permissible, conditionally permissible, and prohibited activities based on evolving scientific capabilities and societal values. These guidelines emphasize specialized scientific and ethics oversight for activities such as genome editing of embryos, chimera formation, and organoid research. They advocate for informed consent, equitable access, and transparency to support ethical integrity in both research and clinical translation. By evolving in line with both science and societal perspectives, the ISSCR Guidelines help bridge the gap between innovation and moral responsibility (Lovell-Badge et al., 2021).

Result

Genome-edited human pluripotent stem cells (hPSCs) provide a highly controllable platform to dissect monogenic diseases and screen for therapeutic modifiers. By generating isogenic cell lines, researchers can investigate the specific role of genetic variants in β-cell dysfunction. Such models have proven critical for understanding diabetes pathophysiology and optimizing therapeutic responses. These insights facilitate the development of genotype-guided treatments in precision medicine strategies (George et al., 2021). Human

pluripotent stem cell models have proven essential in unraveling the diverse phenotypic outcomes of FBN1 mutations in Marfan syndrome. More than 50 hPSC lines have been developed to mirror patient-specific skeletal and cardiovascular anomalies. These models bridge developmental biology with translational cardiology by enabling gene–phenotype correlation. They serve as predictive tools for patient stratification and individualized therapeutic planning (Aalders et al., 2024).

Stem cell models are at the forefront of simulating complex human disorders like Huntington's disease and spinal muscular atrophy. Through directed differentiation into neurons and cardiomyocytes, these systems allow detailed analysis of disease progression in a human cellular context. CRISPR-based gene editing further enables the dissection of causative mechanisms and the testing of targeted treatments. Such models facilitate both phenotypic characterization and preclinical drug screening (Sterneckert et al., 2014). Stem cell-based models not only replicate tissue architecture in 3D cultures but also capture disease-specific pathophysiology with remarkable accuracy. The integration of CRISPR/Cas9 with stem cell biology has empowered researchers to model rare diseases and test pharmacologic responses in vitro. These platforms are highly scalable, rendering them ideal for high-throughput applications. As a result, they accelerate the translation of molecular insights into therapeutic innovations (Bai, 2020).

The integration of stem cell biology and genetic engineering has become one of the fundamental pillars of modern biomedical science. Advances in basic sciences have enabled a deeper understanding of the genetic regulation of cellular differentiation, while innovations such as CRISPR, iPSCs, and organoid technologies allow for more precise disease modeling and the development of personalized therapeutic strategies. In clinical practice, stem cell and gene-editing technologies offer promising approaches across a wide spectrum of conditions, from hematological disorders to neurodegenerative diseases.

However, these advancements also bring significant ethical, legal, and social debates. Therefore, progress in this field requires not only technical success but also ethical responsibility, safety protocols, and public awareness.

In the future, stem cell and genetic sciences may offer groundbreaking solutions in areas such as personalized medicine, artificial organ production, and the prevention of hereditary diseases. These developments, supported by scientific foresight, interdisciplinary collaboration, and ethical oversight, represent one of the most transformative advances in human health.

This book chapter aims to provide an educational and research-oriented resource that covers both the theoretical foundations and therapeutic applications of stem cell biology and genetic engineering.

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