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Review Article

Integrating 3D culture systems and gene editing technologies for precision medicine in hematological malignancies

Sariga Dhanasekar[1], B. Meenakumari[1]*, Bargavi Kathirvel[2], K. Chandramouleeswari[2]

[1]Department of Molecular Pathology, Institute of Child Health and Hospital for Children, Madras Medical College, Chennai, Tamil Nadu, India
[2]Department of Pathology, Institute of Child Health and Hospital for Children, Madras Medical College, Chennai, Tamil Nadu, India

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***Correspondence:**
Dr. B. Meenakumari,
E-mail: hemmeena@gmail.com

ABSTRACT

Haematological malignancies comprise a diverse group of life-threatening systemic diseases in the blood, bone marrow, and lymphoid tissues, and their progression is strongly influenced by a supportive tumour microenvironment that drives proliferation and drug resistance. Drug development and personalised treatment using patient-derived cells are a tedious and expensive process, often conducted in 2D cell culture, which has many uncertainties and limitations due to poor mimicry of the microenvironment, cellular mechanisms, and morphological changes. Animal models also fail to fully replicate human pathobiology and are time-consuming. Alternatively, three-dimensional (3D) in vitro tissue modelling techniques can replicate the complex microenvironment, resembling the morphology, cell-cell interactions, and extracellular matrix (ECM) through methods like organoids, spheroids, tumoroids, and scaffold-based cultures. High-throughput drug screening in edited 3D cultures allows analysis of tumour interactions in haematological malignancies, for example, assessing gene-mediated drug resistance in AML within the marrow niche, depending on specific myeloma plasma cell cultures and their interactions with mesenchymal stem cells, along with susceptibility to CAR-T regulators and clinical translation in patient-derived therapies. The clinical translation of 3D culture and gene-editing–based in vitro models in leukemia, lymphoma, and multiple myeloma predicts cellular interactions with the tumour microenvironment, thereby enabling targeted treatment options for patients. This review highlights current progress in integrating gene editing with 3D culture system and emphasises their potential to enhance personalised drug discovery and preclinical testing in haematological malignancies.

Keywords: 3D culture, Hematological malignancy, Gene editing, Tumour interaction, Extra-cellular matrix, Tumour microenvironment

INTRODUCTION

Hematological malignancies, or blood cancers, represent one of the most prevalent cancer groups in economically developed regions of the world. These are categorised by detecting cancer cells in the blood, lymph nodes, or Bone marrow because these cancers begin in blood-forming tissue.[1] Each type of hematologic malignancy is a complex and diverse disease, consisting of various biological subtypes. This diversity leads to multiple treatment approaches and varying survival outcomes.[2] Also, haematological malignancies are carried out whether, in the germline or somatic mutation, the germline is the mutation that occurs or develops in the sex cells, so mutation occurs in fertilization and passes from one generation to another, whereas somatic mutation occurs in isolated certain cells.[3,4]

The leads of inadequate treatment promote an increase in the mortality rate and efficiency of therapeutic strategies because every year more patients suffer due to malignancies.[5]

Leukemia is defined as the abnormal production of leukocytes (i.e., the subpopulation of Hematopoietic stem cells (HSPCs)) in the origin of cells, which are either myeloid or lymphoid, and based on the proliferation; it is classified as acute or chronic. So, the complete subtypes and predominant ones are acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), involving the myeloid lineage; acute lymphoblastic leukemia (ALL); and chronic lymphocytic leukemia (CLL), involving the lymphoid chain. Acute leukemia (AL) is a fast-progressing type of cancer affecting the bone marrow and blood, caused by the overproduction of immature white blood cells, known as blasts.[6] ALL occurs by the proliferation of immature lymphoid cells and AML by the proliferation of myeloid cells in the blood. In paediatrics, ALL is found to be more common, and AML is found in any age group, but more prevalent within adults.[7] CLL by the proliferation in monoclonal lymphoid cells and Chronic myeloid leukemia (CML) arises from a reciprocal translocation between the BCR gene on chromosome 22 and the ABL1 gene on chromosome.[9] This genetic rearrangement forms a fusion gene that produces a dysregulated tyrosine kinase on chromosome 22, known as the Philadelphia (Ph) chromosome. Both AML and CML are extremes from myeloid progenitors and myeloblasts. Less common variants, such as mature B-cell leukemias, T-cell leukemias, and NK cell-related leukemia's, originate from mature white blood cells.[6] More than adults, malignancies are more common in children, over 40% of all new cancer diagnoses, leukemia is the most prevalent type because it can arise from any blood lineage.[2]

More than 90 lymphoma subtypes are present, which are groups of malignant neoplasms of lymphocytes that include lymphatic tissue, bone marrow or extranodal sites and are broadly classified as non-Hodgkin and Hodgkin lymphoma. Increase in risk of lymphoma aetiologies by genetic, infectious, and inflammatory.[8] Hodgkin's lymphoma is more prevalent in young adults with unique histologic, immunophenotypic and clinical features. It consists of two subtypes: classical Hodgkin's lymphoma (cHL) and nodular lymphocyte-predominant Hodgkin lymph. Majority of Hodgkin lymphoma cases are falls under classical Hodgkin lymphoma (cHL), while the other subtype, nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), makes up approximately 5% of cases.[9] Around 25% of patients either relapse or fail to respond to initial therapy. While conventional treatments achieve a cure rate of 90% in early-stage Hodgkin lymphoma (HL), the cure rate drops to 70% for those with advanced-stage disease using standard approaches.[10]

Non-Hodgkin lymphoma (NHL) encompasses a diverse group of diseases with varying clinical and biological behaviours, ranging from slow-growing (indolent) to fast-progressing (aggressive) forms. Indolent subtypes include follicular lymphoma (FL), marginal zone lymphoma (MZL), cutaneous T-cell lymphoma (CTCL), small-cell lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL), and lymphoblastic lymphoma. Aggressive subtypes include diffuse large B-cell lymphoma (DLBCL), peripheral T-cell lymphoma (PTCL), and mantle-cell lymphoma (MCL), with DLBCL being the most common. DLBCL alone represents approximately 30% of all NHL diagnoses in adults.[11]

The most complex haematological malignancy primarily by abnormal growth and proliferation of clonal plasma cells, predominately in the bone marrow, is called multiple myeloma (MM)—proliferation of clonal plasma cells, which increase the secretion of immunoglobulins and non-functional proteins.[12] Multiple myeloma must be carefully differentiated from related plasma cell disorders, such as monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM), and other similar conditions.[13] Multiple Myeloma progresses rapidly, which leads to the shrinkage of normal cells in the bone marrow, which induces osteoclast immigration, and messes with Mesenchymal stem cell (MSCs) differentiation into osteoclasts, which end up in abnormal bone resorption.[14]

The development of single-targeted therapy faces huge difficulty due to the intra- and inter-heterogeneity of malignant tumours and cancer cells. Various models are required to study how the cancer cells evolved, the cancer initiation process, drug resistance, recurrence, and relapse of the disease diversity. Cell-based models are made in in-vitro models, which are known as 2D culture, a monolayer adherent culture used for a long period. To exactly mimic the biological behaviour of tumor cells and the mechanism that leads to therapeutic escape and drug resistance, 3D cultures are more appropriate than the 2D cell culture.[1]

Concurrently, novel 3D cell culture models, such as spheroids and organoids combined with multiplex genome editing advancement makes it possible to dissect complex gene networks and signalling pathways that underlie tissue homeostasis and disease. These models are effective tools for high-throughput drug screening, synthetic lethal interaction mapping, and validation of new therapeutic targets.[16] Overall, the union of gene editing technologies and 3D model systems closes the gap between in vitro studies and in vivo models, providing a strong and flexible platform for mechanistic investigation, drug discovery, and personalized medicine applications.

This review aims to provide a detailed overview of haematological malignancies, distinguished by their molecular differentiation and gene editing technologies. It explores advanced development in targeted therapy through gene editing and 3D culture that mimics the tumour microenvironment to study the disease progression while addressing key challenges such as disease relapse, drug resistance, and tumour heterogeneity. Additionally, it highlights the role of these innovations in enhancing preclinical studies, improving drug screening, and developing personalised treatment strategies. This is intended to reveal a deeper understanding of the diagnosis and treatment of haematological malignancies.

3D CULTURE SYSTEM IN HAEMATOLOGICAL CANCER RESEARCH

New drugs have evolved using high-throughput screening to establish potential drug molecules. These processes are based on the 2D cell culture technique, in which the cell culture takes place on a flat plastic surface. However, there are some disadvantages to 2D cell culturing, in which the exact physiological conditions are not represented. There is a high risk of contamination in the media used for culture, which leads to high maintenance, and there is no certainty. In tumour cells, some communications are not well represented like cell-cell interaction and cell-external environment, which leads to polarity loss. The limitations of 2D cell culture have been overcome by 3D cell culture, which replicates the in vivo condition and physiological state of the culture used.[17]

3D cell culture method that resembles the physiological environment and more relevant cell models are produced, which is the primary benefit of 3D culture over a 2D culture. 3D cell culture has high complexity and a steady environment in the tumour structural environment and well-kept flow of body fluids like blood, plasma, and urine.[18] It is the best technique to mimic the complete cellular interaction in the tumor microenvironment. Drug testing is usually conducted using animal models, which pose ethical concerns; however, these issues can be mitigated by the 3D cell culture method.[19]

Conversely, 3D systems, such as spheroids and organoids, demonstrate a high degree of maturation and functionality. These structures are often generated by the formation of stem cell aggregates known as embryoid bodies.[20] This result stems from more in vivo-like signalling and biophysical gradients, as well as cell–cell contacts, that can impact the various transcriptional networks and metabolomic pathways within the cell.[21,22] Various techniques in 3D culture description and application are summarized in Table 1.

Table 1: Application of 3D models and its techniques.

3D Model	Various techniques for construction	Common types	Description	Applications	Key references
Spheroids	Hanging drop method, non-adhesive plates (ultra-low attachment), rotary bioreactors	Multicellular tumor spheroids (MCTS), scaffold-based spheroids, scaffold-free spheroids	Aggregates of cancer or normal cells forming spherical structures; mimic cell-cell and cell-matrix interactions; simple and reproducible	Cancer biology and hypoxia studies, drug screening and toxicity testing, angiogenesis and invasion studies	Edmondson et al, 2014 (Assay Drug Dev Technol) Costa et al, 2016 (Trends Pharmacol Sci)
Organoids	Stem cell–derived (ESCs, iPSCs, ASCs), embedded in ECM (Matrigel, collagen), growth factor–guided differentiation	Intestinal, liver, kidney, brain, lung, patient-derived organoids (PDOs)	Self-organizing 3D structures derived from stem cells; recapitulate organ architecture and function; long-term expansion possible	Developmental biology, disease modeling (genetic disorders, infections), precision medicine and regenerative therapy	Lancaster and Knoblich, 2014 (Science) Clevers, 2016 (Cell)
Tumoroids	Derived from patient tumor tissue (PDOs from cancer biopsies), embedded in ECM scaffolds (Matrigel), supported by growth factor cocktails	Patient-derived tumoroids, co-culture tumoroids (with immune cells, fibroblasts)	Organoid-like cultures established directly from tumors; preserve tumor heterogeneity, microenvironment, and genetics	Cancer biology and tumor heterogeneity studies, drug screening and therapy response prediction, immunotherapy testing (co-culture with T/NK cells)	Sachs et al, 2018 (Cell) Drost and Clevers, 2018 (Nat Rev Cancer)

Spheroids

Cells aggregate on non-adherent platforms, forming the 3D structure of tumor growth and enhancing the tumor microenvironment. 3D cell cultures are self-assembled and composed of cancer cell lines or patient-derived cells. Spheroids facilitate tumor cell progression, increase drug resistance, and improve the evaluation of anti-cancer treatments. Additionally, they allow for a better understanding of the tumor cell phenotype in response to chemotherapy.[23] Since spheroids are derived from cancer cell lines, they have the potential to form a tumor-like microenvironment that can induce metastasis, promote cell-cell and extracellular matrix interactions, facilitate nutrient transport, and regulate growth factors. This occurs because they can accurately replicate the core properties of solid human tumors.

Organoids

Organoids are 3D models derived from and induced by hematopoietic stem cells (HSCs) that are organ-related or from pluripotent stem cells, either embryonic or adult. They have the capacity for multilineage differentiation into various cell types, allowing them to mimic *in vivo* 3D models of specific organs. For example, lung-derived stem cells can be used to study lung physiology and morphology. Organoids can replicate patient-specific phenotypes from primary patient-derived normal or tumor tissues with high accuracy, enabling the study of individual drug responses for patient-specific targeted therapy in precision medicine.[24]

Tumoroids

A 3D culture fully preserves the tumor's genetic profile, allowing for the study of each patient's unique tumor pattern. The cells used in these cultures are derived from patient-derived stem cells, primary tumor biopsies, or circulating tumor cells. Because it retains the molecular characteristics of the original tumor, a 3D culture provides a more specific model for studying tumor heterogeneity and personalised treatments, such as responses to chemotherapy or targeted therapy. Additionally, it aids in identifying novel drug targets.[25]

GENE EDITING IN 3D CULTURE SYSTEMS

Genome editing of stem cells represents an important contribution to our knowledge of basic biologic principles but is also entering its first decade of therapeutic application. The intersection of gene editing tools with 3D cell cultures represents a paradigm shift that has revolutionized modeling of hematologic malignancies for basic research and therapy. CRISPR/Cas9 genome editing enables the precise modulation of oncogenes, tumor suppressors, and epigenetic modifiers in biologically relevant 3D spheroids and organoids, solving important issues of two-dimensional cultures.[26] In contrast to conventional 2D monolayer cultures, 3D systems better replicate the oncologic principles of tissue architecture, oxygen gradients, and cellular interactions.[27] Genome editing of leukemia and multiple myeloma by the CRISPR/Cas9 system in 3D scaffolds has uncovered patterns of gene sensitivity and resistance that remain invisible in traditional cultures.[28,29]

Patient-derived organoids maintain tumour genomic, transcriptomic, and epigenomic properties, supporting the functional validation of candidate targets in the context of individual patients.[16] High-throughput CRISPR screens coupled with 3D culture models also facilitated the application of synthetic lethality analyses, including microenvironment-based factors, focusing on chromatin modifiers, mitochondrial regulators, and apoptosis.[26] CRISPR knockout phenotypes and drug sensitivity are changed or influenced by signalling from the niche in 3D co-culture systems that include stromal and immune cells.[30] Because of this, using gene editing techniques to study and develop drugs for precision therapy in haematological cancers must be performed on 3D culture systems to have a strong mechanistic basis and offers a highly translatable platform.

EXPLORING TUMOUR IMMUNE INTERACTION IN 3D CULTURES

Basic research using 3D culture involves the derivation of stem cells from healthy individuals to study developmental processes and the generation of 3D cell culture in order to study cell-cell interactions, external stimuli, and stress signalling. In patients, stem cells are obtained and then cultured in an extracellular matrix to enhance differentiation and growth. The cells are then stored in biobanks for pathogen analysis, genomic, and metabolic analyses, as well as screening for disease-related genetic variants for disease modelling. Data from patients can thus be used to develop targeted drugs, cell therapies, and genetic engineering strategies.

The immune microenvironment plays a critical role in the progression and response to therapy of haematological malignancies. Conventional 2D models lack the complex interplay between immune and malignant cells that mediates immune evasion and resistance to therapy. In AML, three-dimensional co-culture systems of leukemic blasts combined with bone marrow-derived stroma and immune cells express higher levels of PD-L1, CXCL12, and anti-apoptotic genes such as BCL2 and MCL1, which confer immune evasion and chemoresistance.[30,31] Similarly, in ALL, three-dimensional hydrogel and microfluidic systems disclose the way leukemic cells interact with stromal components in order to suppress T-cell cytotoxicity through immune checkpoint upregulation, including PD-L1 and TIM-3, closely mirroring in vivo marrow interactions.[32] In CML, 3D spheroid cultures of $CD34^{+}$ stem-like and immune effector cells show increased resistance to tyrosine kinase inhibitors and reduced NK-cell-mediated cytolysis, further underlining their potential to predict patient-specific therapeutic outcome.[33] When integrated into high-throughput 3D formats, these immune–tumour co-cultures allow comprehensive immunotherapy screening-such as CAR-T/CAR-NK cells, checkpoint inhibitors, and cytokine-blocking strategies-enabling simultaneous evaluation of cytotoxicity, immune infiltration, and cytokine modulation across hematologic malignancies to be performed.[34] Thus, 3D culture systems recapitulate the structural and functional complexity of the leukemic niche and provide physiologically relevant tools to investigate tumor-immune dynamics and test next-generation immunotherapies.

HIGH-THROUGHPUT DRUG SCREENING USING 3D MODELS

High-throughput screening using miniaturised 3D systems with AML, ALL, or MM cell lines embedded in matrices

such as Matrigel, alginate, collagen, PEG hydrogel, or matrix-free spheroids enables the identification of drug candidates and their resistance mechanisms. These models are highly predictive of physiological responses to drugs, as they reflect the cell-cell and cell-matrix interactions, thus improving the translational predictive value of preclinical screening. The design parameters for such models involve determining the model size to accurately reconstitute pH, oxygen, and soluble factor gradients, together with the presence of a proliferative outer layer, hypoxic cores, and necrotic zones, among other features, while choosing the composition of the ECM and stroma influences the screening of novel anti-tumour agents.

Collateral sensitivity within 3D cell culture refers to the selective killing of drug-resistant cells rather than their sensitive parental counterparts. The ECM's physicochemical and mechanical characteristics, such as stiffness and cellular remodelling, profoundly impact drug response and therapeutic outcomes. For instance, Shin and Mooney encapsulated AML cell lines in alginate hydrogels and demonstrated that increased matrix stiffness enhanced proliferation in certain lines, whereas softer matrices conferred drug resistance but heightened sensitivity to AKT inhibitors, findings that were further validated in vivo using xenograft mouse models.[28] While 3D models generally exhibit higher drug resistance than 2D cultures due to restricted drug diffusion, specific agents may demonstrate heightened efficacy depending on their mechanism of action.[29]

The advantages of 3D cultures in drug discovery are their support for multicellular cocultures, including tumour, stromal, and immune cells, which provide a physiological tumour microenvironment. The tumour stroma consists of cancer-associated fibroblast (CAFs), immune cells, endothelial cells, mesenchymal stem cells, adipocytes, and the components of the ECM. It promotes tumor progression, drug resistance, immune evasion, and angiogenesis by secreting growth factors, cytokines, and chemokines. This has been shown by Assaraf et al, Hanahan, Coussens, Kitaeva et al, Hirata and Sahai.[35-38]

Traditionally, HTS for evaluating CAR-T cell cytotoxicity has relied on 2D assays. However, Chen et al, in 2018, developed a high-throughput system based on 3D hanging spheroids, allowing efficient screening of CAR-T and CAR-NK cells for the destruction of tumor spheroids in different cancers.[34] Also, this platform enables parallel testing of anti-cancer drugs within 3D models. This is well exemplified by azacitidine and cytarabine, both showing greater resistance in 3D cultures, as evidenced by higher BCL2 expression, $p<0.05$.[39]

Overall, the integration of immune-tumor interactions, ECM biophysics, and high-throughput 3D platforms marks a significant step forward toward physiologically relevant, patient-specific drug discovery and immunotherapy testing in hematological malignancies.

CLINICAL TRANSLATION OF 3D CULTURE AND GENE EDITING IN HEMATOLOGIC MALIGNANCIES

3D models form an integral part of study for blood cancers, with the ability to throw light on the tumour microenvironment (TME) and its role in mediating cell-cell interactions. The 3D models can replicate to some level the role of TME that mediate tumor development and response to therapies.

Leukemia in vitro models

Multiple niche environments and high cellular heterogeneity make it challenging to replicate the leukemia tumor microenvironment due to which fewer attempts have been made to replicate these models in vitro. Models often exploit non-contact interactions between different cell types by the use of trans-well inserts or by use of 3D hydrogels or scaffolds with ECM proteins to co culture different cell lines and obtain different compartments that provide a more physiologically relevant spatial organization and cell communication.[40,41] Complicated interactions between cells such as endothelial cells, mesenchymal stem cells (MSCs), immune cells and ECM has been the cornerstone in disease progression. Due to variability in disease features and niche compositions across different subtypes of leukemia, a single model cannot represent them all accurately. For instance, B cell infiltration especially in CLL is associated with disease progression and drug resistance. Existing research shows that even though advanced models such as 3D scaffolds, hydrogels and micro fluids are making models more physiologically relevant, replication of patient derived behavior remains difficult. Notable examples have been summarized in the Table 2.

Multiple myeloma in vitro models

Multiple myeloma cells reside in the bone marrow niche where cell-ECM adhesion via hyaluronic acid and fibronectin activates survival pathways that block apoptosis such as NF-κB, PI3K/AKT.[42] The bone marrow niche includes hematopoetic stem cells, non-hematopoietic cells such as MSCs, osteoblasts, endothelial cells and non-ECM components like collagens, glycosaminoglycans, proteoglycans.[43] The primary cells of MM fail to expand in standard ex vivo 2D cultures demonstrating the necessity of micro environmental cues in mediating viability and proliferation. ECM mediated adhesion activates CAM-DR particularly through β1 integrin signaling that enhances survival against proteasome inhibitors. MSCs secrete IL-6, 1L-10, and CX3CL1 that upregulates STAT3 and MAPK pathways to promote proliferation.

CX3CR1 and CXCR4 enhance BM homing and disease progression. Thus, the in vitro models need to be expanded to include ECM proteins to reproduce adhesion mediated drug resistance (CAM-DR); MSCs to recreate paracrine

IL-6/1L-10 survival signalling; 3D or dynamic culture conditions to mimic BM mechanical and diffusion characteristics as the dynamic mechanical cues preserve quiescence and drug tolerance more than 2D models.[44] Notable examples have been summarized in the Table 3.

Table 2: Overview of 3D in vitro microenvironment models and key findings in leukemia.

Study	Model system	Key findings
Torres-Barrera et al	Co-culture of CML stem/progenitor cells with endothelial cells	Direct contact with endothelial cells maintains LSCs and progenitors, supporting their survival and quiescence
Svozilová et al	RGDS-functionalized hydrogel for CLL + stromal cells	Scaffold supported long-term stromal/cell line survival, but patient CLL cells survived only briefly, highlighting difficulty in maintaining primary CLL
Bray et al	3D PEG-heparin hydrogel with endothelial cells + MSCs + AML cells	AML cells localized near the vascular network and were more drug-resistant in 3D co-culture; CXCR4 inhibition disrupted interactions and reduced protection
Ma et al	Microfluidic BM niche model for ALL	Demonstrated niche-dependent proliferation, quiescence (especially in the endosteal region), and drug resistance; SUP (resistant) cells remained drug-resistant in the model
Barbaglio et al	3D scaffold + rotating bioreactor for CLL	Showed stromal cell–dependent infiltration, microenvironment-regulated protein expression, and cell mobilization after drug (ibrutinib) treatment
Cavelleri et al	Comparative analysis of CML cell lines (K562, LAMA-84, KCL-22)	Demonstrated distinct differences in signaling profiles, proliferation rates, and TKI responses. Highlights the importance of using multiple CML models to improve translational relevance for personalized therapy
Lica et al	Culture of very small embryonic-like stem cells (VSEL-SCs) to generate leukemic stem-like cells (L-SLCs)	Showed that primitive VSEL-SCs can be transformed into L-SLCs, enabling mechanistic study of early leukemogenesis and malignant stem-cell evolution
Ladikou et al	3D bone-marrow-mimicking scaffold co-culture (primary AML + mesenchymal stromal cells + endothelial cells)	Identified CD44-FAK signaling as a key axis supporting AML adhesion and survival. Inhibition of this pathway reduced chemoresistance, demonstrating clinical therapeutic relevance
Lindacher et al	3D scaffold-based tri-culture (CLL cells + bone-marrow stromal cells + T cells)	Spatial positioning influenced drug response: core-localized CLL cells exhibited higher resistance and altered stromal signaling. AP-1 complex identified as a regulator of niche-mediated resistance

Table 3: Overview of 3D in vitro microenvironment models and key findings in multiple myeloma.

Study	Model type/ materials	Cell types used	Microenviro -nment features replicated	Key experimental findings (specific data)	Drug response observations
Trujillo et al, 3D dynamic microsphere culture	Polymer microspheres coated with hyaluronic acid (HA) or fibronectin (FN); cultured on rotating plates to maintain suspension	MM cell lines: RPMI8226, U226, MM1.S	Reproduces CAM-DR by promoting integrin-mediated adhesion to HA/FN under dynamic shear	Microsphere–ECM interaction increased MM cell proliferation versus suspension controls. Adhesion promoted shift toward drug-resistant phenotype.	Bortezomib resistance increased up to 20-fold (cells continued proliferating despite treatment). No resistance developed in standard suspension culture. Dexamethasone showed reduced sensitivity only in ECM-coated conditions.
Ferrarini et al, RCCS™ bioreactor	Rotating bioreactor maintaining low shear and gas	Patient-derived MM explants,	Preserves native tissue architecture,	MM tissue remained viable for 14 days with structural	Drug testing with proteasome inhibitors preserved patient-

Continued.

Study	Model type/ materials	Cell types used	Microenviro -nment features replicated	Key experimental findings (specific data)	Drug response observations
for ex vivo MM tissue	exchange for intact fragments	containing MM cells + vasculature + stromal cells	including arterioles and cell–cell contacts	preservation of niche architecture (confirmed histologically).	specific therapeutic sensitivity profiles, enabling clinically relevant ex vivo drug response evaluation.
Spelat et al, MSC-loaded thermo-responsive hydrogel	p(GMMA)-b-p(HPMA) hydrogel loaded with MSCs placed in a transwell above MM cells	MSCs (in hydrogel) + MM cell lines in suspension	Recreates paracrine MSC signaling without direct contact	MM co-cultured with MSC-hydrogel upregulated CX3CR1 expression (receptor promoting BM homing and survival). Also observed upregulated IL-6 and IL-10, confirming inflammatory protective signaling.	Co-culture led to significantly increased MM proliferation vs. monoculture. Demonstrates that MSC-secreted cytokines alone are sufficient to enhance MM survival.
Wu et al, Coaxial extrusion 3D-bioprinted BM cavity model	Hollow bone-marrow–like tube (outer shell = bone-like matrix; inner core = MM region). Bioink: GelMA + alginate + PEGDA + nano-HAp	MM1.S cells alone, or MM1.S + HS5 stromal cells, also tested with patient MM cells	Mimics cortical bone boundary + stromal-rich medullary core; supports long-term cell organization	MM cells + HS5 stromal co-culture showed spatial aggregation similar to in vivo BM niches. Patient-derived MM cells survived 7 days in 3D, versus ≤5 days in 2D.	Bortezomib IC50 increased 3-fold versus 2D, and 1.7-fold versus MM-only 3D. Co-treatment with Tocilizumab (IL-6 receptor blockade) improved BTZ killing and reduced stromal adhesion-mediated resistance.

Lymphoma in vitro models

86% of NHL arises from B lymphocytes and 14% arise from T cells or NK cells. NHL pathologies are strongly influenced by the dynamic interactions between tumor cells and malignant components such as immune cells, stromal cells like MSCs, blood vessels and the ECM. The interaction of MSCs and NHL cells contribute to an immunosuppressive, anti-inflammatory micro environment, enhancing tumor proliferation and chemo resistance. Only a few HL and NHL models exist due to difficulty in recreating complex TME cell-cell interactions, poor in vivo survival of cell types and challenging co-culture conditions. Bahlmann et al developed a biomimetic cryogel that supports tumour associated macrophage (TAM) invasion made of hyaluron-gelatin-fibronectin. Screening identified five inhibitors (Marimastat, Batimastat, AS1517499, PD-169316, Ruxolitinib) that reduced TAM colonization or repolarized TAMs toward pro-inflammatory phenotypes.[45] Mastini et al developed a microfluidic perivascular model of large cell anaplastic lymphoma using a commercially available DAX-1 microfluidic chip. The device consisted of two lateral channels lined with endothelial cells (HUVECs) to recapitulate vascular structures and a central channel representing the perivascular niche through which lymphoma cells were introduced. Co-culture of lymphoma cells with endothelial cells in this system resulted in a marked reduction in the cytotoxic efficacy of the tyrosine kinase inhibitor crizotinib. Mechanistic investigation revealed that this protective effect was mediated through activation of the CCL19–21/CCR7 signaling axis, highlighting the key role of endothelial–tumor crosstalk in supporting lymphoma cell survival. This model therefore underscores the influence of the vascular niche in promoting drug resistance and persistence of lymphoma cells during therapy, and demonstrates its utility for evaluating microenvironment-targeted treatment strategies.[46]

Integration of gene editing and 3D culture into patient derived therapies

The combination of gene editing technologies and 3D culture models in patient-derived therapies marks an important step forward in treating blood cancers.[47] By using patient-specific cells grown in realistic 3D environments, personalized gene editing outside the body can be performed to improve the effectiveness and precision of treatments.[48] Edited cells, such as blood stem cells or T cells, are then reintroduced into patients as either autologous or allogeneic therapies. This allows for customized treatment strategies. This method not only predicts clinical outcomes better but also enables quick testing of treatment options, connecting laboratory breakthroughs to individualized patient care.

Integration of CRISPR-cas9 gene editing with 3D disease models

sgRNA is used to direct Cas9 to a target DNA sequence by the adaptive immunity of certain bacteria to induce double stranded breaks, which are repaired by non-homologous end joining or homology directed repair. The combination of 3D organoid systems and CRISPR-Cas9 has enabled modelling of relevant disease physiologically, providing cultural heterogeneity and tissue architecture not found in 2D cultures. Patient-derived organoids can retain genomic and transcriptomic characteristics of the original tumor, allowing for personalised drug testing and oncological studies.[16] In haematological malignancies, CRISPR-based screens have been instrumental in the identification of epigenetic modulators, mitochondrial regulators like MTCH2 and post translational modifiers like CMAS, SLC35A1, NANS, GNE as contributors to cell survival in leukemia and also the basis of drug resistance. This modality allows for synthetic lethality testing and drug gene interaction profiling identifying vulnerabilities that can be exploited for targeted therapy.

ZFN-based gene editing in T-cell therapies for leukemia

Adoptive T cell therapy is a promising modality for cancer, but high-avidity tumour-specific cells are uncommon and hard to expand. The TCR gene transfer allows redirecting patient T cells against tumors. While this seems promising, the downsides include the competition introduced between the endogenous and induced TCR chains. Mispairing between endogenous and exogenous α/β chains may occur, resulting in reduced therapeutic TCR expression and formation of new unintended TCRs with unpredictable or autoreactive specificities, raising safety concerns. Provasi et al conducted a study with the objective of completely eliminating the endogenous TCR expression using ZFNs following which high avidity tumor specific TCR could be introduced via lentiviral transduction to generate pure correctly paired high functioning tumor specific T cells.[49] ZFNs were used to knock out the TRBC1/2 genes, generating TCR/CD3-null T cells which were successfully expanded using IL-7 + IL-15. These edited cells were then transduced with a WT1-specific TCR, restoring uniform CD3 expression without chain mispairing. The engineered T cells displayed higher receptor density, greater antigen-specific cytotoxicity, and stronger functional avidity against $WT1^{+}$ targets. They also showed no off-target alloreactivity, improving safety compared to conventional TCR-T cells. Overall, complete TCR replacement enhances potency, specificity, and suitability for universal adoptive T cell immunotherapy.

CHALLENGES AND LIMITATIONS

Although the gene editing technology and 3D culture techniques in hematological malignancy are advanced, several significant technical, biological and logistical challenges exist, which limits their general usage. Though they mimic the tumour microenvironment in hematological malignancy, but the primary challenge is replicating the complex TME of hematopoietic tissues, variety of stromal and immune cells, and a functional vascular network. By increasing the pathogenic interactions or mechanisms, identification of relapse of malignant cells will be made possible, which in turn will facilitate novel therapeutic drug development for relapsed patients. As mentioned earlier, though it mimics the tumour microenvironment, it has its limitations; it was more expensive and time-consuming compared to 2D cultures and needs to be developed to bridge the preclinical-to-clinical gap. In 3D culture development, organoid technology faces challenges to overcome from its infancy stage when compared with established cell lines and animal models. The gene editing tools, like RNAi, are only knockdown systems with low efficacy, prone to off-target effects, whereas transposons are stable gene expression systems using a cut-and-paste mechanism and sometimes produce changes in host cells due to the gene of interest affecting active genes. One major limitation when using spheroids in preclinical studies is related to the lack of standardized and straightforward assays for imaging, analysis, quantification, and automation in pharmaceutical drug screening. When compared to 2D cultures, only a few protocols and technologies are available for compound effect assessment in 3D systems, making standardization of data difficult to achieve.[50,51]

Moreover, the predictive value of spheroids concerning drug efficacy remains so far uncertain because the correlation between spheroid responses and clinical outcomes is still unproven, according to Gunness et al in 2013 and Fang and Eglen in 2017.[52,51] However, studies on new analytical methodologies and technologies for spheroids have increased rapidly over the past few years.[53]

Lenti virus has the adaptation to move the viral DNA via the nuclear pore complex into the host cell's nucleus without requiring the cell to go into division. This limitation is usually exploited in the immune cells or the non-dividing cells, which are pretty tough to infect.[54] With both of these retro- and lentiviruses, the integration usually occurs in the sites that are transcribing actively, which, in turn, can adversely impact the expression of the host genes. Moreover, the viral vectors both have a limit on carrying a DNA insert about 8 kb, covering most of the cDNAs but not all of them.[55] The other approaches to the delivery involved the parameters such as voltage, which were pretty optimal for each of the devices and cell types and were relatively expensive. Extensive pilot testing was required for all. Liposomes are formed by the lipofection, which utilises the lipofectamine or other lipid molecules. These liposomes are introduced into the cells with encapsulated DNA.[56]

The latter technique is quite simple and usually very effective in many types of cells. However, the transgene expression is usually transient, and sometimes the lipofection can compromise the viability of the cell.

CONCLUSION

Gene editing technologies and 3D model culture systems together mark a significant change in cancer research and targeted therapy development. Gene-editing tools like CRISPR allow for precise manipulation of oncogenic pathways. This enables a deeper understanding of tumor biology and helps identify actionable molecular targets. At the same time, 3D culture platforms, which include organoids and spheroids, provide relevant models that closely mimic tumor structure, diversity, and interactions within the environment. When combined, these technologies create strong platforms for modeling cancers, testing therapy weaknesses, and predicting how patients will respond more accurately than traditional methods. Although challenges remain, including delivery efficiency, off-target effects, high cost and the need for standardized 3D model protocols, the combined promise of these innovations' places them at the forefront of future precision oncology. In the end, their continued improvement could speed up the development of more effective, personalized, and less harmful cancer treatments in the field of hematological malignancy.

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