Advances in Ex vivo Cell Screening Methodologies for Clinical Therapy Guidance to Leukemic Patients

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Abstract

Advances in liquid biopsy handling and processing have reinvigorated interest in ex vivo screening methods for drug discovery and as an emerging feature of patient care in the field of hematological cancers. Treatments based on ex vivo assays are gaining traction since they have demonstrated new benefits using approved drugs to treat leukemia and associated cancers. Various unique assay platforms, specifically designed to predict therapeutic response in leukemic patients within a clinically actionable time frame, have shown high accuracy, reproducibility and high-throughput potential and promise to serve as a decision support system for therapeutic management of multiple hematologic malignancies.

Keywords: Hematologic malignancy; Ex vivo tissue culture; Tumor microenvironment; Clinical therapy guidance; AML

Introduction

Blood cancers are becoming more manageable but remain incurable. Despite a growing armamentarium of effective next-generation agents, choice of therapy, especially in relapse or acquired resistance, still relies almost exclusively on clinical acumen. Integrating strategies from multi-disciplinary research efforts is critical for the translation of advances in ex vivo protocols to successful personalized medicine. It is becoming possible to assess the sensitivity or resistance of an individual's own bone marrow or blood fraction to a panel of drugs or drug combinations [1,2]. Such attempts aim to match the best treatment to each patient, and equally importantly, protect a patient from detrimental treatment, which is the ultimate goal of any precision medicine endeavor. This mini review briefly discusses specific techniques that can be applied to achieve better outcomes for patients with leukemia and associated cancers, that are particularly difficult to reliably model ex vivo.

Discussion

High-throughput screens that better mimic the tumor microenvironment are important for generating clinically relevant data. In the past, the inability to sufficiently recapitulate the native physiological environment sufficiently by accounting for the presence of bone marrow, stroma, platelets, plasma etc. prevented ex vivo drug sensitivity testing from becoming clinical and predictive. One report described a patient-derived primary acute myeloid leukemia (AML)-enriched cell suspension being co-cultured on a confluent monolayer of mesenchymal MS-5 cells at 3% O_2, 37 ^oC, 5% CO_2 in a humidified chamber [3]. Within bone marrow pockets where leukemic cells primarily originate and reside, the oxygen levels are 0-5% compared to ~21% in air. These conditions were found to be better suited for maintenance of self-renewal of leukemia-initiating cells over a period preferred to observe meaningful experimental outcomes. These cells were cultured in the presence of a five-factor cytokine cocktail of c-Kit lig and, interleukin-3, interleukin-6, erythropoietin, and granulocyte colony-stimulating factor as essential supplementary factors. Myeloid-restricted mouse engraftment was observed in all samples, indicating that the residual normal hematopoietic stem cells that could have been present within leukemic samples did not out-compete the AML cells during ex vivo culture.

Khoo et al. [4] have developed a novel approach to evaluate patient drug response ex vivo using patient blood-derived circulating tumor cell (CTC) cultures in an integrated micro fluidic system incorporating micro-fabricated micro-wells. Their assay comprises custom-designed tapered micro-wells that allow for robust CTC capture and expansion without pre-enrichment. Cultures are maintained as multi-layered clusters, which better reflects the in vivo state of a tumor. The underlying principle of the CTC cluster assay is the concentration of patient-derived blood cells as companion cells in a hypoxic chamber to mimic in vivo tumor conditions. Combination of these factors permits expansion of CTCs without pre-enrichment procedures or additional growth supplements, allowing the potential of maintaining most CTC subpopulations. The integrated device showed robust performance with consistent generation of concentration gradients under various flow rates and input reagent concentrations. The use of standardized micro-wells via micro-fabricationis important to

allow even distribution of cells in each micro-well, leading to the formation of clusters with consistent morphology. Tapered micro-wells are also preferred over conventional cylindrical micro-wells for cluster formation. This could be due to the inclination of the walls in tapered micro-wells, which allowed collected cells to settle in the center, forming a single cluster (instead of multiple small aggregates) to allow close interaction and communication between blood cells and CTCs. Incorporation of acoustic liquid dispensing technologies within such a system can further aid novel studies on the cumulative action of existing drugs when used in combination for therapy.

Another study demonstrated the value of a dynamic rotary cell culture system (RCCSTM) bioreactor-based method that takes advantage of the unique milieu generated by simulated microgravity conditions, characterized by low shear and turbulence, optimal oxygen and nutrients delivery and waste removal [5]. This bioreactor system allows culture of hematological cancer samples from patients, preserving the proper topographic and functional interactions between tumor cells and the hosting microenvironment. In such conditions, tissue culture could be maintained for up to two weeks, thus substantially extending the duration (few days) achieved with currently available methods. The model could be further implemented to recapitulate more closely physical features of the bone marrow, and in particular hypoxia, that are more crucial while evaluating leukemic samples.

Conclusion

More numbers of studies with larger sample sizes will be helpful in standardizing a set of protocols that can be robustly applied for the purpose of delivering clinical therapy guidance to all subsets of leukemic patients in the future.

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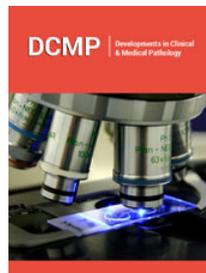
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