Short Communication

MSC.sensor: Capturing cancer cell interactions with stroma for functional profiling

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1. Introduction

Ex vivo drug testing of primary tumor cells from individual patients is being evaluated in clinical trials to guide therapeutic decisions for patients with resistant disease. However, primary tumor cells are generally difficult to maintain viable ex vivo in monoculture. Coculture of primary tumor cells with representative components of the in vivo microenvironment can maintain metabolic homeostasis and viability of primary tumor cells to extended periods. The complexity of the multicellular systems involved is nicely recapitulated using organoids derived from human induced pluripotent stem cells.

Two-dimensional models have been used to assess the impact of stromal cells in co-culture with malignant cell lines, but it remains challenging to specifically measure the viability of primary tumor cells in co-culture. Conventional viability assays based on metabolic activity (such as ATP present) or cytotoxicity (such as lactate dehydrogenase release) do not distinguish tumor cells from stromal cells. In addition, assays that can specifically measure the tumor cells (for example, flow cytometry) require laborious steps with limited scalability. Bone marrow mesenchymal stromal cells (MSCs) maintain the viability of primary acute lymphoblastic leukemia (ALL) cells in ex vivo culture and influence the response to anti-leukemic agents. Even in these simplified models it remains difficult to follow the fate of leukemia cell subpopulations depending on their contact with the microenvironment. Here, we engineer a stroma-based biosensor for specific detection of tumor cells in a coculture model of bone marrow. Using this system, we performed drug response profiling to explore promising drug combinations to target high-risk leukemia subtypes in a protective context.

2. Results

To specifically monitor the live ALL cells that interact with MSCs in coculture, we engineered hTERT-immortalized MSC cells to express a CD19 binding synthetic Notch receptor (CD19-SynNotch-TetRVP64) that controls expression of an integrated reporter cassette TetO-BFP (MSC.CD19sensor.BFP, Fig 1A). When coculture with CD19+ ALL cells, the membrane tethered transcription activator TetRVP64 is released and drives the expression of a BFP reporter in MSC cells (Fig 1B). As the synthetic Notch receptor only responds to surface tethered ligands, the reporter of the MSC.sensor can only be induced by CD19 bound beads but not soluble CD19 (Fig 1C). Thus, measurement of the reporter signal from MSC.sensor provides a way to monitor the viable ALL cells in contact with cocultured stromal cells in situ.

While imaging-based analysis can collect many spatially and temporally resolved parameters, a bioluminescence-based readout is...
more convenient for quantification in high-throughput viability assays. Therefore, we engineered a bioluminescence reporter of MSC.sensor (MSC.CD19sensor.Lum) using the NanoLuc luciferase fused to a PEST domain [8]. This reporter yields signals with higher sensitivity and dynamic range than fluorescence signals. Single cell clones of MSC.sensor cells were generated and displayed a similar capacity to support the viability of ALL patient-derived xenografts (PDXs) in coculture as the parental MSC population (Fig. 1D). Coculturing of a fixed number of MSC.CD19sensor.Lum cells (2,500 / well in 384 well plates) with an increasing number of ALL PDXs cells yielded a linear increase of bioluminescence signal up to a ratio of ALL/MSC of 4 (Fig. 1E). Thus, the bioluminescence signal from MSC.CD19sensor.Lum can be used as a surrogate readout for viable ALL cell count to scale functional screening.

Although MSCs usually have a higher tolerance to chemotherapy than primary ALL cells, their physiological status should not be neglected while profiling with drugs in cocultures. To evaluate the impact of drugs on the MSC.CD19sensor.Lum cells, we measured the bioluminescence signal (driven by CD19 bound beads) and viability (ATP-based assay) of MSCs treated with a selection of anti-cancer agents that are routinely included in contemporary functional precision.

**Fig. 1.** MSC.CD19sensor for monitoring of ALL cells in coculture. (A) Schematic of the implementation of a CD19 SynNotch receptor in MSC cells. (B) MSC.CD19sensor.BFP-mediated displaying of ALL PDX cells in coculture measured by flow cytometry (left) and microscopy (right). (C) Reporter signal from MSC.CD19sensor.BFP cells exposed to increasing amounts of CD19-conjugated Dynabeads. (D) Viabilities of PDXs from 10 ALL patients after 3 days in monoculture or coculture with parental MSC population, single cell clones from MSC.CD19sensor.BFP or MSC.CD19sensor.Lum. Cell viability was measured by 7-AAD staining and flow cytometry. (E) MSC.CD19sensor.Lum-mediated displaying of ALL PDX cells in coculture measured by bioluminescence. Increasing amount of PDXs cells from 6 ALL patients were seeded together with 2500 MSC.CD19sensor.Lum cells per well in a 384-well-plate. The bioluminescence signals were acquired at 24 h, 48 h and 96 h after seeding. (F) Comparison of the bioluminescence signal decay from MSC.CD19sensor.Lum and ALL cell count measured by flow cytometry (7-AAD negative) after treatment with 1uM Venetoclax in 2 PDXs. PDXs from 2 ALL patients were seeded together with MSC.CD19sensor.Lum at a ratio of 4:1 one day before Venetoclax treatment.
synergy to be further developed as safe and effective treatment options. We considered Venetoclax as an ideal backbone for exploring syndromes using the MSC.CD19sensor.Lum coculture with ALL PDXs (Fig. 1F). A progressive decay of the reporter signal matched the decrease of the PDX cell count detected by flow cytometry (7-AAD staining) upon 24 h treatment (Fig. 1F).

Combination drug screening is a valuable strategy to identify drug synergy to be further developed as safe and effective treatment options for cancer patients [9]. This approach usually requires high-throughput measurement of cell viability in large-scale combinational matrixes [10]. We explored candidate drug synergies targeting high-risk ALL subtypes using the MSC.CD19sensor.Lum coculture with ALL PDXs (Fig. 2). We considered Venetoclax as an ideal backbone for exploring syndromes with other compounds because: (1) Venetoclax has favorable pharmaceutical properties and efficacy against several high-risk ALL cohorts including TCF3-HLF and MLL-rearranged ALL [11–13]; (2) Combination of Venetoclax with other therapeutics achieves sustainable remission in many tumor types [14]; and (3) Genome-wide CRISPR analysis of chemotherapy–gene interactions in ALL cells uncovered that sgRNA targeting of BCL2 scored broadly as a common target whose inhibition could enhance the response to many chemotherapy agents [15].

We established a high-throughput pipeline for combinational drug screening of Venetoclax with 12 therapeutic agents in high-risk ALL cells (Venetoclax plus screen, Methods) (Fig 2, Supplementary Fig S2). Venetoclax showed the strongest synergy with the MCL1 inhibitor S63845, which is in consistent with the functional redundancy of BCL2 and MCL1 in cell death control and endorses the clinical development of this approach in ALL therapy [16] (Fig 3A). Our profiling also revealed potent synergistic combinations of Venetoclax with L-asparaginase and the XPO1 inhibitor Selinexor consistently across all tested PDXs (Figs 3A and 3B). Combinational treatment in a TCF3-HLF cell line (HAL-01) and a TCF3-PBX1 cell line (697) recapitulated these synergies in monoculture (Supplementary Fig S3). The first-in-class nuclear transporter inhibitor Selinexor may represent a promising therapy for ALL patients and is currently in phase I/II clinical trial for pediatric relapsed/refractory acute myeloid leukemia (AML) patients (Clinicaltrials.gov identifier NCT04988894). To confirm the on-target effect on XPO1 we performed a CRISPR knockout of XPO1 and a sgRNA competition assay in HAL-01 and 697 cells. We found that cells expressing XPO1 sgRNAs were rapidly outcompeted by non-transduced cells shortly after infection, which was further pronounced under Venetoclax treatment (Fig 3C). Given the evidence of the synergy between Venetoclax and Selinexor in AML, diffuse large B-cell lymphoma and multiple myeloma [17], this combination may represent a paradigm of synthetic lethality in many hematologic malignancies.

3. Discussion

Although cell lines have been widely used in functional studies and preclinical drug screenings, cell lines have adapted to grow without niche support and do not represent the molecular complexity of the disease at presentation [3]. Experimental models that more faithfully reflect the primary disease may facilitate the development of next generation therapeutics. We and others have established coculture systems to support primary ALL ex vivo enabling functional profiling using imaging-based approaches [5,6]. While imaging records many morphological features of the cells, it generally requires complex analytical pipelines with elaborate software for quantification which require costly infrastructure [18]. Furthermore, inter-sample heterogeneity of leukemia cell morphology confers additional complexity for data analysis by automated imaging. Engineering of biosensor MSCs, which detect ALL cells that interact through CD19 as a surrogate biomarker confers several advantages: (1) Specific detection of leukaemic CD19 blasts, which is particularly valuable for bone marrow samples with low leukaemia cell infiltration (detection of a small subpopulation of cells without additional cell sorting steps); (2) Objective readout that captures the total amount of leukaemia cells in contact in coculture; (3) Label-free and fast assay, which is suitable for high content exploration studies. Although CD19 is dispensable for ALL cells survival [19] and binding with CD19 antibody does not impact ALL cell viability (Fig 1D) [20], engagement with cell surface molecules may rewire downstream signaling and should be considered when designing sensors for other biomarkers in a new model.

We demonstrate the application of MSC.sensor platform to facilitate the exploration of clinically effective combination therapies in ALL. Using a 384-well plate holding 2,500 MSC / well, it is practical to detect 100 – 10,000 ALL cells / well. This format can be further miniaturized, for example by using a 1536 well plate or microfluidic systems to spare primary material. Since the MSC.sensor “reflect” the presence of ALL

![Fig. 2. Schematic of the combinational screen in ALL-MSC coculture in 384-well plates. (A) Illustration of dispense pattern of tested compounds creating 7 × 7 dose combinations in a 384-well plate. ALL cells were cocultured with MSC.CD19sensor.Lum cells for 24 h then treated with combinations for 72 h. Bioluminescence signals from MSC.CD19sensor.Lum cells were acquired at the end of treatment and transformed into normalized inhibition (%) over the dose matrix. (B) Dose ranges of selected compounds tested in Venetoclaxplus screen. (see methods section for more details).](attachment:image.png)
through contact, this system may also constitute a feasible platform for pooled genetic screens interrogating non-cell-autonomous determinants supporting tumor survival [21].

4. Methods

4.1. Plasmids

The anti-CD19 SynNotch-TetRVP64 receptor vector pHr_PGK antiCD19_synNotch_TetRVP64 (Addgene #79,126) was described previously [7]. The BFP reporter vector pHr_TetO_tBFP_PGK_mCherry was derived by subcloning the CTS-TRE3GV-TagBFP cassette from pLVX TRE3 G BFP vector (Addgene #128,070) into the pHr_Gal4UAS_tBFP_PGK_mCherry vector (Addgene #79,130) using KflI and EcoRI sites. The NanoLuc-PEST reporter vector pHr_TetO_NLucPEST_PGK_mCherry was derived by swapping the BFP cassette of pHr_TetO_tBFP_PGK_mCherry vector with NLuc-PEST cassette (PCR from the Pci 9.0 vector (Addgene #74,229)) using MluI and EcoRI sites.

4.2. Lentivirus production and transduction

Lentiviruses were produced by transfecting HEK 293T cells with transfer plasmids, pSAX2 (Addgene #12,260) and pCMV-VSV-G plasmids (Addgene #8454) in a ratio of 5:3:2 using polyethylenimine (Polysciences, 24,765–2). Viral supernatants were collected 30 h after transfection and used for transduction immediately.

4.3. Generation of MSC.CD19sensor cells

Human hTERT-immortalized primary bone marrow MSC were provided by Dario Campana (St Jude Children’s Research Hospital, Memphis, Tennessee) and maintained in RPMI-1640 medium (Sigma-Aldrich, R0883) supplemented with 10% fetal bovine serum (Sigma-Aldrich), penicillin (100 u/ml)-streptomycin (100 mg/ml), L-glutamine (2 mM) and 1 μM hydrocortisone. For lentiviral transduction, fresh virus’ supernatant was added directly to the cells. Viral media was replaced with normal growth media 24 h after infection. MSC cells were first infected with pHr_PGK_antiCD19_synNotch_TetRVP64 vector encoding the anti-CD19 SynNotch receptor and a myc-tag and sorted by anti-myc A647 (cell-signaling #2233) staining. The MSC stably expressing the anti-CD19 SynNotch receptor were then infected with either pHr_TetO_tBFP_PGK_mCherry vector or pHr_TetO_NLucPEST_PGK_mCherry vector and sorted by mCherry to generate bulk populations of MSC.CD19sensor.BFP or MSC.CD19sensor.Lum cells. Single-cell clones from MSC.CD19sensor.BFP and MSC.CD19sensor.Lum cells were generated by seeding single cells in 384-well plates. Reporter signal induction of all single cell clones was detected by coculture with ALL cells and monitored by flow cytometry or bioluminescence assay.

4.4. Patient samples and patient-derived xenografts

Patient samples were collected from different countries within the International BFM Study Group (I-BFM-SG) as described previously [5]. Informed consent was given in accordance with the Declaration of
Helsinki. Approval for experiments with human samples in the mouse xenograft model was obtained from the ethics commission of the Canton Zurich (approval number 2014–0383). All animal studies were carried out in accordance with relevant guidelines and regulations and ARRIVE guidelines. Primary ALL cells were recovered from cryopreserved samples and transplanted intraperitoneally or intravenously into 8–12 week old NOD.Cg-PkdcsidclI2gutm1Wij/SzJ (NSG) mice. Leukemia progression was monitored by flow cytometry with rat anti-mouse CD45 (eFluor450, clone 30-F11, REF 48–451–82, eBioscience) and mouse anti-human CD19 (PE, clone HIB19, REF 302,208, BioLegend) antibodies. ALL cells recovered from spleens of NSG mice were used for molecular characterization in vitro and in vivo experiments.

4.5. ALL-MSC coculture and reporter assay

MSC or MSC.CD19sensor cells were co-cultured with ALL PDX cells in fixed ratios. The BFP reporter signals from MSC.CD19sensor.BFP cells were measured by flow cytometry and the bioluminescence reporter signals from MSC.CD19sensor.Lum cells were measured by a luminescence plate reader after adding a passive lysis buffer containing Furimazine directly in the coculture.

4.6. Drug screen assay

For drug testing, ALL PDX cells were maintained in coculture with MSC.CD19sensor.Lum in a physiologically relevant medium (Plasmax, Ximbio), which mimics the metabolic and physiological profile of human plasma [22], supplemented with 2.5% fetal bovine serum. 10,000 PDX ALL cells and 2,500 MSC CD19sensor.Lum (clone 8) cells were seeded together in 30ul medium per well in a white 384-well-plate. After a 24-hour incubation, drugs or the vehicle were added using a drug printer and incubated for 72 h. At the end of treatment, a Furimazine reaction mixture (2.5ul of PBS, 7.5ul of 5x Passive lysis buffer (Promega) and 0.04ul of 20 mM Furimazine (AOBIOUS)) was added directly to each well and incubated at room temperature in the dark for 10 min. The bioluminescence signals were read by a luminescence plate reader. Viability of ALL cells was determined by the normalized bioluminescence signals. For the combinational screen (VenoctolaxPlus screen), the dose range of Venetoclax was set from 0.3 mM to 100 mM covering the IC50 values of the majority of the tested ALL PDX. The bioluminescence signals were acquired at the end of treatment and the synergistic score was calculated based on the Zero interaction potency (ZIP) model [23].

4.7. CRISPR knockout and sgRNA competition assay

A TCF3-HLF+ ALL cell line (HAL-01) and a TCF3-PBX1+ ALL cell line (697) were firstly infected with a Cas9 vector (pl40C.PGKintron.Cas9 Green [13]). Cells with stable Cas9 expression were infected with a sgRNA vector (sg.shuttle.RFP657 [24]) coding the sgRNAs targeting XPO1 (sg.XPO1: AGTGAGCTCTCAAAAAACGT; sg.XRPO1 (2): TCACACCAGCAACTCAGTT) or a scramble sequence (sg.SCR: GTAGCGAACGTGTCCGGCGT). In the sgRNA competition assay, the multiplicity of infection (MOI) of the sgRNA vectors was controlled to achieve a transduction of ~50%. The infected populations (RFP657+) were monitored by flow cytometry at different timepoints with or without Venetoclax treatment.

Declarations of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data underlying the results are available as part of the article (see Supplementary Data) and no additional source data are required.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.slasd.2023.08.003.

References