PromoCell®

Isolation of Tumor Associated Macrophages (TAM) from Fresh Tumor Tissue

Application Note

Background

Tumor-associated macrophages (TAM) represent an important constituent of the tumor microenvironment (TME). After being educated by cancer cells, TAM adopt an anti-inflammatory, pro-tumor and pro-metastatic M2-like phenotype promoting progression of the disease [1]. So far, circulating precursor monocytes were thought to represent the main cellular origin of TAM, however there is increasing evidence that tissue-resident macrophages may also be recruited, especially with increasing malignancy of the tumor [2].

MMP2/9, B7-H4, STAT-3, CD163, and CD206 have been used as putative markers for classification of TAM within different macrophage subsets. Indeed, due to the high plasticity and heterogeneity of these cells, a consensus marker profile for TAM has not been established so far [3].

A high local TAM infiltration within the tumor generally represents an indicator of a poor prognosis. However, the high plasticity of the macrophage as a cell type also poses new opportunities by means of the targeted reprogramming of TAM to a pro-inflammatory / anti-tumor phenotype in the context of novel therapeutic interventions [2]. Therefore, TAM have now become another important target in anti-cancer research.

Using the PromoCell Primary Cancer Culture System (C-28081), TAM can be isolated from primary tissue samples of solid tumors as non-proliferating adherent cells and can be cultured for at least two weeks.

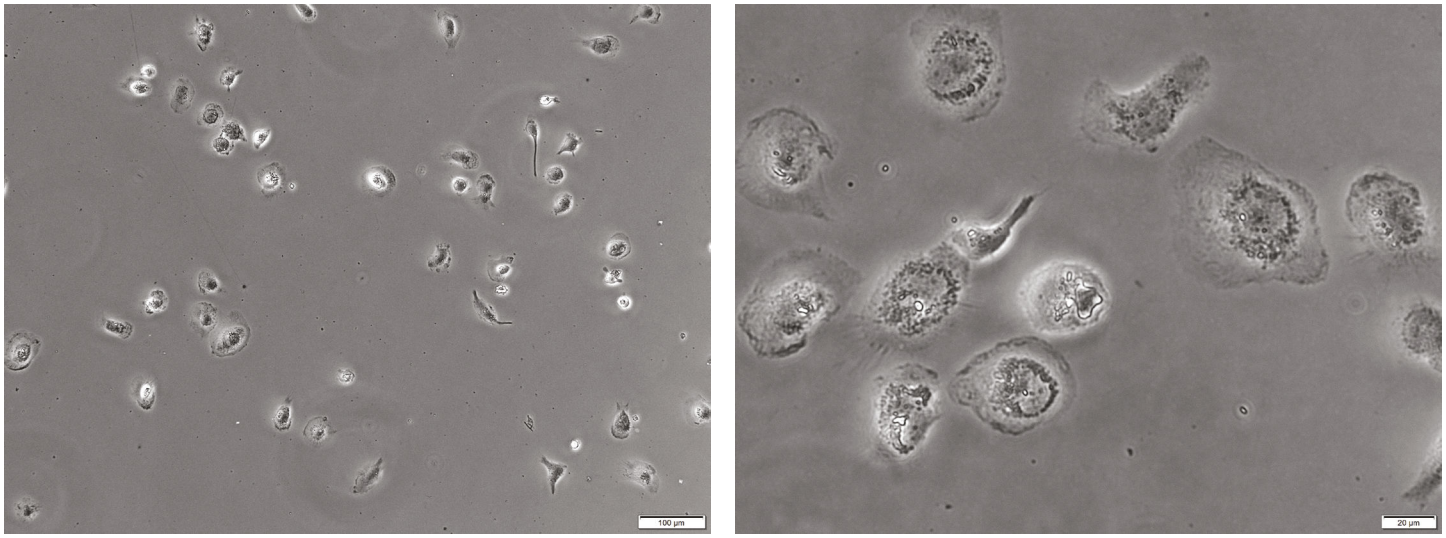

Figure 1. Phase contrast images of Tumor Associated Macrophages (TAM) isolated from primary human tumor tissue. *The TAM were isolated according to the given isolation protocol from human tumor tissue, a lung metastasis of an unknown primary tumor. The cells show the characteristic "fried-egg"-phenotype of M2-like macrophages. Images were taken 4 days after isolation (magnification left 100x, right 400x).*

TAM Isolation Protocol

I. Materials

- Fresh tumor tissue (0.2–3 grams; ≥ 1 gram is optimal)
- Hanks Balanced Salt Solution (HBSS) with Ca^{2+}/Mg^{2+} without Phenol Red (C-40370)
- Primary Cancer Culture System (C-28081)
- M-CSF from E.coli or HEK cells
- Gentamicin (50 mg/ml stock)
- Phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+} (C-40232)
- Accumax (e.g. Sigma #A7098) for tissue digestion
- Scalpel / forceps / scissors
- Cell strainers of descending size down to 70 µm (e.g. 400 / 100 / 70 µm)
- Tilt-roll-shaker, rotary mixer or comparable
- Tissue culture treated 24 well-plates (also refer to protocol step II.9)

II. TAM isolation procedure

The Primary Cancer Cell Medium D-ACF allows for the isolation and culture of tumor associated macrophages (TAM) while reliably preventing stromal overgrowth.

Keep in mind that the cells do not proliferate and that the yield of isolated TAM from primary tumor tissue may be low depending on the specific sample and type of tumor.

Note: The following isolation protocol is a basic method suitable for a wide range of solid tumors. However, alternative methods for isolation of tumor-derived single cells may be adequate and advisable for demanding tissues regarding their successful homogenization.

1

Wash and weigh the tumor tissue (day 0)

Remove visible residues of healthy tissue from the tumor. Place the tumor sample in a tube and wash twice with a generous amount of PBS and vigorous shaking. Then weigh the tumor tissue in a pre-tared sterile petri dish.

Note: The tumor tissue should be as fresh as possible and stored in HBSS at 2 to 8 °C immediately after surgical removal. Tissue up to 6 hours old is optimal for isolation purposes. However, successful isolations have been accomplished from tumor samples as old as 24 hours. Keep in mind that recently applied chemical or radiation therapy may affect the isolation results.

2

Homogenize the tumor tissue (day 0)

Place the washed tumor sample on the lid of a petri dish. Add a small volume (1–2 ml) of Primary Cancer Cell Medium D-ACF to the tumor tissue and dissect it into small pieces using a scalpel. Homogenize the tissue to a "slurry" or into small pieces of approx. 1 mm^3 by additionally mincing the tissue chunks using the scalpel. Avoid attrition of the tissue.

3

Wash the homogenized tumor tissue (day 0)

Transfer the homogenized tumor tissue to a 50 ml tube using forceps. Add 10x the volume (w/v) of PBS and vortex or mix vigorously. Let the tissue pieces settle for 2 minutes and then aspirate the supernatant. Repeat if there is still a lot of blood/debris observable. Aspirate as much as possible of the PBS without losing the tissue.

Note: If there is floating homogenized tissue, use a sieve, e.g. 400 µm, for separating the washed, homogenized tissue from the washing buffer.

4

Perform the enzymatic digest of the tumor tissue (day 0)

Resuspend the tissue pellet in Accumax solution at a concentration of 20 ml per gram of tumor tissue. Incubate at room temperature (20-25°C) with gentle but constant mixing, e. g. by a tilt-roll mixer at 50 rpm. Digest until the solution becomes distinctly turbid. Depending on the type of tissue, this is typically the case after approximately 30–60 minutes. A 45 minute incubation is a good starting point.

Note: Do not digest the tissue longer than necessary and never digest for longer than 60 min ince this may significantly compromise cell viability. Always perform the digestion reaction at room temperature and consult the Accumax manual for instructions on proper storage and handling.

5

Remove tissue residues from the sample (day 0)

Let the remaining tissue pieces settle down for 2 minutes. In order to obtain a single cell suspension, progressively filter the turbid supernatant using cell strainers of descending pore size down to 70 µm, e.g. 400 µm > 100 µm > 70 µm.

Note: Discard the remaining tissue pieces.

6

Dilute the sample with medium (day 0)

Dilute the single-cell suspension at least 1:1 with Primary Cancer Cell Medium D-ACF or a suitable buffer, e.g. PBS / Albumin / EDTA. Use a higher dilution ratio if the solution is still viscous.

7

Obtain the isolated single cells (day 0)

Pellet the cell suspension for 15 minutes at 350 x g at room temperature and carefully aspirate the supernatant without disturbing the cell pellet. Leave a small amount (20 – 50 µl) of supernatant left.

Note: Use 15 ml conical tubes and strictly adhere to the given centrifugation speed and time for best results in the centrifugation step!

8

Resuspend the obtained single cells (day 0)

First, resuspend the cell pellet by snipping with your fingers. Then resuspend the cells in Primary Cancer Cell Medium D-ACF supplemented with 250 ng/ml M-CSF per 1ml medium per gram of isolated tumor tissue. Adapt the amount of medium if necessary, e.g. use 500 µl of medium for 0.5 grams of tumor tissue.

Note: In case cell clumps are observed, which cannot be effectively resuspended, filter the cell suspension once more through a 70 µm cell strainer before proceeding to the next step.

9

Let the cells attach (day 0)

Plate the resuspended cells at approx. 500 µl cell suspension per cm^2 of culture surface and add 50 µg/ml Gentamicin. Incubate overnight at 5% CO_2 and 37°C in the incubator.

Note: Do not use the NCCD Reagent for treatment of the culture surface! Just use standard TC-treated plasticware for TAM-isolation.

Note: 24well plates are well-suited for tumor samples of at least 1 gram. For smaller tissue samples 48well or even 96well plates might be more adequate to match the recommended seeding density and number of samples aimed.

10

Wash the adherent cell fraction (day 1)

By vigorously swirling the tissue culture vessel loosen non-adherent cells and aspirate them. Wash the remaining adherent cells three times with warm PBS by swirling the vessel and aspirating the supernatant.

11

Use the isolated TAM for your experiments or continue their culture (day 1)

The isolated adherent TAM may now be used for your experiments. Alternatively, continue the culture at 37°C with 5% CO_2 using Primary Cancer Cell Medium D-ACF and 250 ng/ml M-CSF and perform regular media changes every 3–4 days.

Note: Make sure to apply some water/buffer to the surrounding wells of the sample(s) in order to limit evaporation during prolonged culture.

Note: The use of alternative culture media instead of the Primary Cancer Cell Medium D-ACF may result in rapid stromal overgrowth of the TAM culture.

Product listing

Product	Size	Catalog Number
Primary Cancer Culture System	250 ml	C-28081
Dulbecco's PBS without Ca^{2+} / Mg^{2+}	500 ml	C-40232

References

1. Lin, Y., J. Xu, and H. Lan, Tumor-associated macrophages in tumor metastasis: biological roles and clinical therapeutic applications. J Hematol Oncol, 2019. 12(1): p. 76.

2. Malfitano, A.M., et al., Tumor-Associated Macrophage Status in Cancer Treatment. Cancers (Basel), 2020. 12(7).

3. Sawa-Wejksza, K. and M. Kandefer-Szerszen, Tumor-Associated Macrophages as Target for Antitumor Therapy. Arch Immunol Ther Exp (Warsz), 2018. 66(2): p. 97–111.

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