In vitro 4T1 and RAW264.7 macrophage culture and subsequent flow cytometric analysis of RAW264.7 macrophage polarization

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SCOPE OF THE METHOD

Alternative method relates to	Human health
Alternative method is situated in	Basic Research
Type of alternative method	In vitro - Ex vivo
This method makes use of	Animal derived cells / tissues / organs
Species from which cells/tissues/organs are derived	Balb-C mouse
Type of cells/tissues/organs	Mammary tumour and macrophages

DESCRIPTION

Method keywords

4T1

mammary cells

tumour

mice

cell culture

RAW264.7
macrophage polarization
flowcytometry

Scientific area keywords

breast cancer
macrophage polarization
triple-negative breast cancer

Method description

The BALB/c-derived - 4T1 mammary tumor cell line and RAW264.7 macrophage cell line were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 □g/ml streptomycin in culture flasks. Harvesting of cultured 4T1 cells was performed using 0.25% trypsin- ethylenediaminetetraacetic acid (EDTA), whereas RAW264.7 macrophages were harvested using a cell scraper. The harvested cells were subsequently washed through centrifugation (805 g for 5 min) and the cell pellets were resuspended in phosphate buffered saline (PBS). Cell numbers were determined through counting using a Bürker chamber. 4T1 mammary tumor cells and RAW264.7 macrophages were cultured either alone (5□×□105 cells in mono-culture) or together (5□×□105 of each cell type in co-culture) supplemented with 1 ml of cell culture medium per well in 24 well plates. The cell cultures were incubated (37 °C, 5% CO2) for 24 h (to examine CHI3L1 and LCN2 secretion) or 96 h (to examine RAW264.7 macrophage polarization) with daily change of the cell culture medium. The harvested cell culture media were spun down (17,000 g) for 10 min to remove cellular debris for further analyses. Cells from 3 wells of 96 h RAW264.7 mono- and 4T1□+□ RAW264.7 co-cultures were harvested using a cell scraper, pooled and washed through centrifugation (805 g for 5 min). The cell pellets were suspended in 2.5 ml FACS buffer (containing PBS, 1% bovine serum albumin (BSA), 2.5 mM EDTA and 0.01% sodium azide) and 100 □l of the cell suspension was plated in a well of a 96 well plate for counting through flow cytometry. Remaining cell suspensions were plated at 100 □l per well in a 96 well plate and the well plate was centrifuged to pellet the cells (805 g for 5 min). To block Fc receptors found on the RAW264.7 macrophages, cell

pellets were subsequently resuspended in FcR blocking reagent (1:10 diluted in FACS buffer) and incubated for 10 min at 2–8 °C. Following centrifugation, cell pellets derived from 4T1□+□RAW264.7 co-cultures were stained for 30 min at 2–8 °C with APC-labeled anti-F4/80 (diluted 1:20 in FACS buffer; clone CI:A3–1) to distinguish RAW264.7 macrophages from 4T1 tumor cells. To allow intracellular staining, the pelleted cells were fixed using BD Cytofix/Cytoperm solution for 20 min at 2–8 °C and permeabilized afterwards by washing twice in 1× BD Perm/Wash Buffer. Cell pellets derived from RAW264.7 mono- and 4T1□+□RAW264.7 co-cultures were stained for 30 min at 2–8 °C with PE-labeled anti-IL-12 (diluted 1:20 in 1× BD Perm/Wash Buffer; clone B211220) or anti-TGF-□1 (diluted 1:40 in 1× BD Perm/Wash Buffer; clone TW7-16B4). Istoype-matched and autofluorescence controls were also included for analyses. Following cellular stainings, cell pellets were washed twice with 1× BD Perm/Wash Buffer prior to analysis with a flow cytometer.

Lab equipment

Biosafety cabinet ;
CO2 incubator ;
Centrifuge ;
Flow cytometer.

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

The BALB/c-derived 4T1 mammary tumor cell line expresses constitutively the firefly luciferase gene and was a kind gift from Prof. Clare Isacke (Breakthrough Breast Cancer Research Centre, London, UK).
This tumor cell line resembles the aggressive phenotype and metastasis seen in human TNBC (estrogen receptor (ER)-negative, progesterone receptor (PR)-negative and human epidermal growth factor receptor 2 (HER2)-negative).

Challenges

Other macrophage-markers were tested (CD38, CD86, EGR2, CD206 and IL-10) to determine polarity (M1/M2) using flowcytometry, but none of these resulted in good signals with positive control samples.

Modifications

We suggest to perform RT-qPCR on the RNA samples of selected genes to distinguish/prove M1/M2 polarity next to flowcytometry analysis.

Future & Other applications

This co-culture method can be applied on a lot of different applications next to reduce (or replace) *in vivo* methods to get a complementary insight on the immune response. But also the monoculture of 4T1 tumour cells can be used in *in vitro* toxicity tests.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Anti-inflammatory signaling by mammary tumor cells mediates prometastatic macrophage polarization in an innovative intraductal mouse model for triple-negative breast cancer. Steenbrugge J, Breyne K, Demeyere K, De Wever O, Sanders NN, Van Den Broeck W, Colpaert C, Vermeulen P, Van Laere S, Meyer E. J Exp Clin Cancer Res. 2018 Aug 15;37(1):191. doi: 10.1186/s13046-018-0860-x.

Associated documents

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