

Supplementary Material

Pleural fluid has pro-growth biological properties which enable cancer cell proliferation

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Supplementary methods

Full culture medium used in cell culture

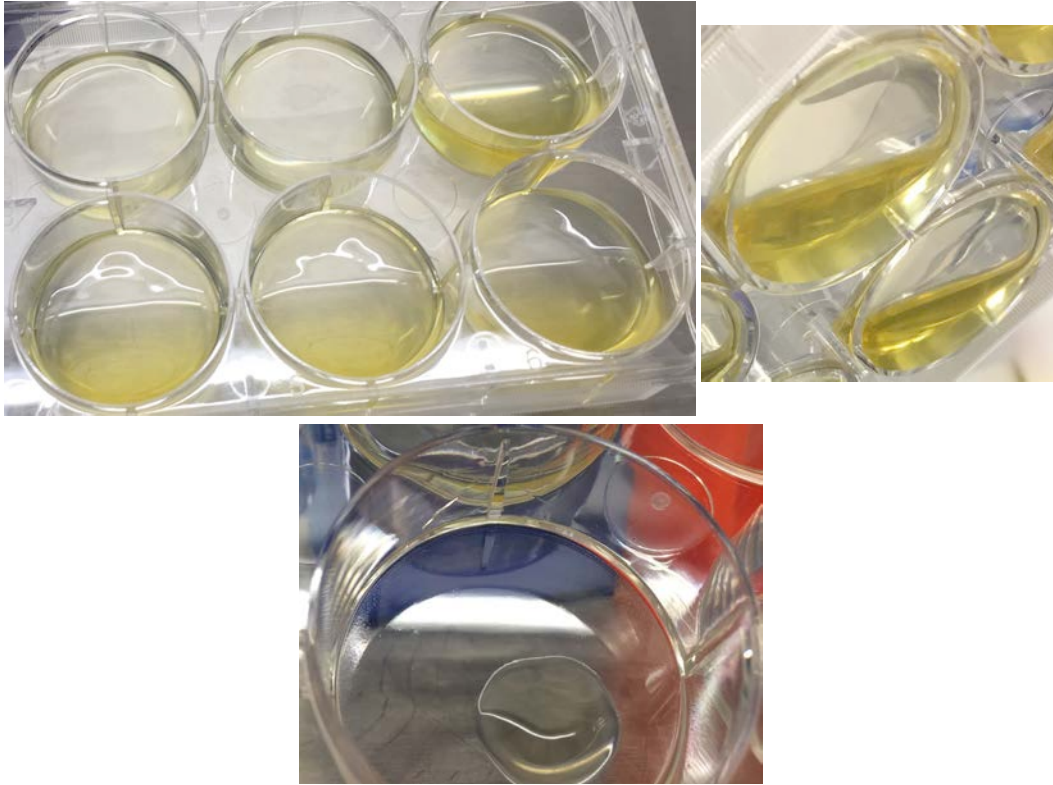
The culture medium used for cell culture was Dulbecco's modified eagle medium (Sigma-Aldrich®, D5671, high (4500mg/L) glucose, without L-glutamine and sodium pyruvate, and with phenol red) with 10% foetal bovine serum (FBS) (Sigma-Aldrich® USA origin, MFCD00132239) and 100 U/ml penicillin, 100mg/ml streptomycin (full medium).

Tendency of pleural fluid to form a gel-like consistency

The pleural fluid tended to adopt a viscous gel-like consistency (Supplementary Figure), and when this happened, it was often not possible to aspirate the fluid with a P1000 pipette. This meant that a number of methods needed to be tested to determine the optimal way of demonstrating the biological effects of pleural fluid, if in fact these were present.

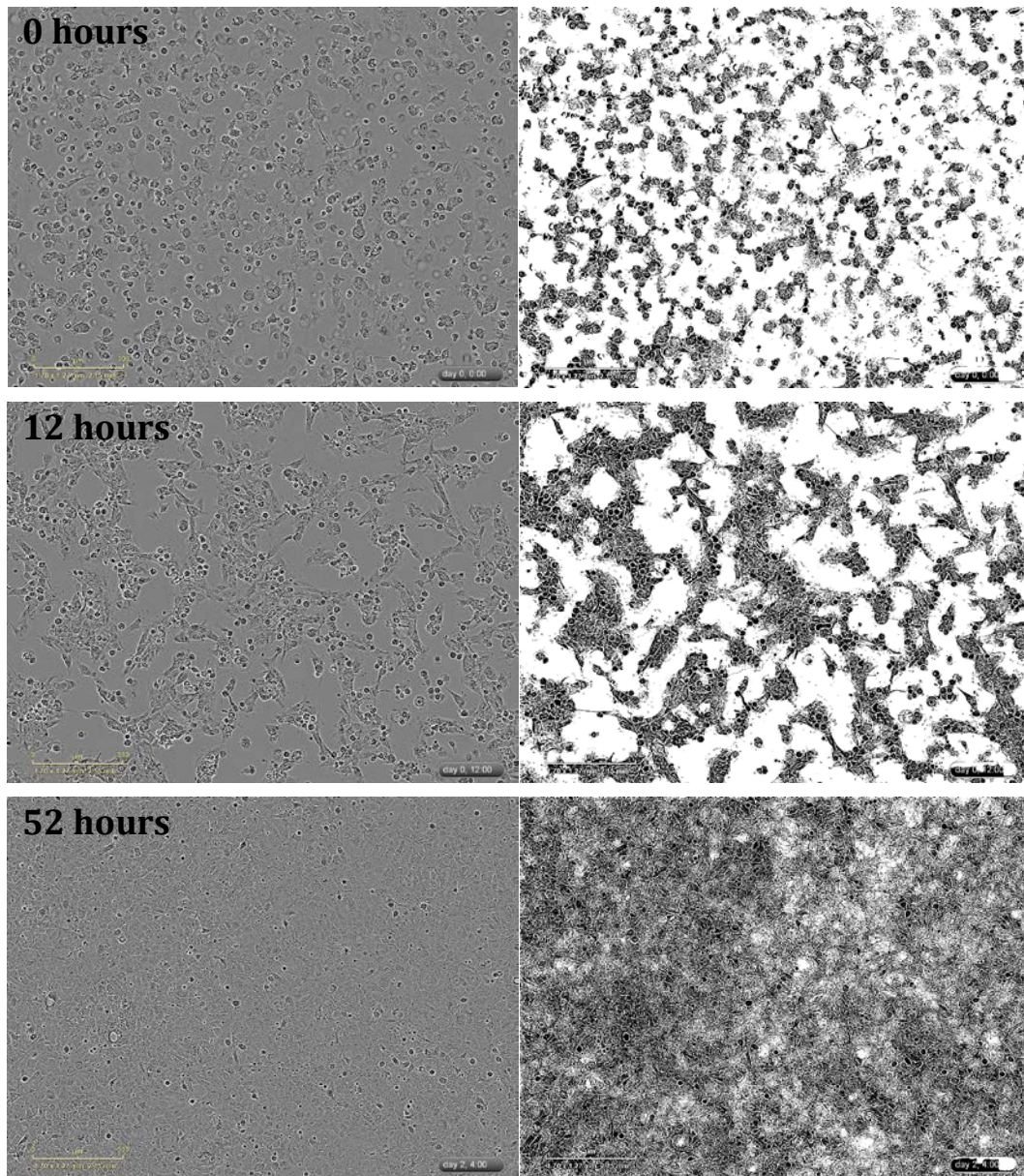
Supplementary Figures

Supplementary Figure 1



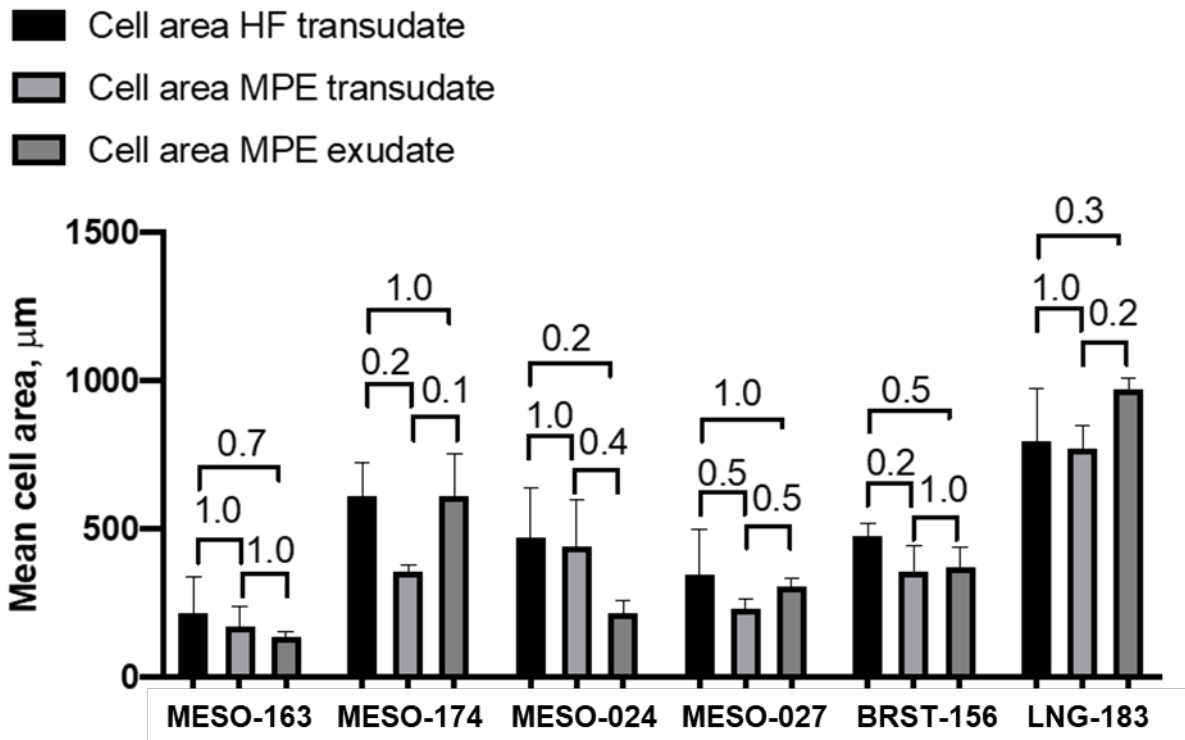
Supplementary Figure 1 shows images of wells within the 6-well plate, containing MPM cells seeded within exudate MPM MPE fluid. Tilting the plate reveals the layer of gelified pleural fluid at the bottom of the wells. Top left: the wells as viewed from above; top right: the wells as viewed from below the plate, bottom centre: a drop of pleural fluid that has adopted a gel-like consistency within the well. *MPE=malignant pleural effusion, MPM=malignant pleural mesothelioma.*

Supplementary Figure 2



Supplementary Figure 2 shows images of MESO-024 cell culture seeded in heart failure transudate pleural fluid. The left sided images are the original images taken by Incucyte® at 10x magnification, and the right sided images are the corresponding images generated by Fiji (ImageJ) version 2.0(11) with manual adjustment of the threshold. Percentage confluence was calculated as 20.2% at seeding (top images), 29.3% at 12 hours after seeding (middle images), and 50.8% at 52 hours after seeding (bottom images).

Supplementary Figure 3



Supplementary figure 3 shows comparison between mean area of cells seeded in the different pleural fluid types. Values above the square brackets indicate the p values obtained (Kruskal-Wallis test) when comparing each mean, and there was no statistically significant difference between size of cells seeded in the different pleural fluid types. *HF*=heart failure, *MPE*=malignant pleural effusion.