The role of ADAMTS5 in Ovarian Cancer Cell Invasion and Migration

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Introduction

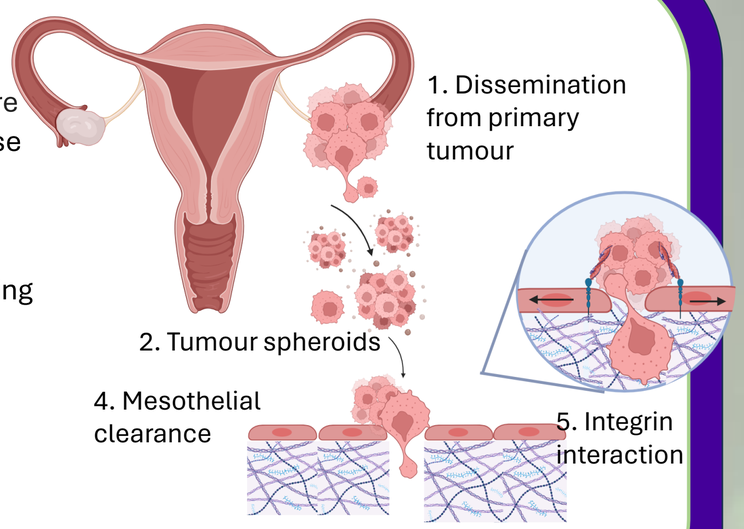
Ovarian cancer is the second most common malignancy after breast cancer in women over 40 and the deadliest reproductive cancer in women. (Stewart, Ralyea and Lockwood, 2019).

Metastasis is the dissemination of cancer cells throughout the body to form tumours at secondary sites. Cancer cells must acquire migratory capabilities for this. Ovarian cancer metastasizes to organs in the peritoneal cavity as individual cells or spheroids, these organs are covered by a layer of mesothelial cells. Metastasis relies on invasion into the mesothelium and interactions with the underlying ECM through integrin binding (Iwanicki et al. 2011).

-Beta1 integrin adheres to ECM protein **fibronectin,** increased expression of which has been seen in ovarian cancer. Integrin binding to the ECM promotes signal transduction leading to mesothelial clearance and invasion of Ovcar spheroids (Caswell et al. 2007).

-Mesothelial cells were believed to act as a mechanical barrier that must be overcome by tumour cells to access the ECM. Research now suggests these cells promote integrin clustering through fibronectin secretion leading to higher migration and invasion, potentially through mesothelial clearance (Kenny et al. 2014).

-ADAMTS5 a matrix degrading enzyme has been shown to correlate with poor prognosis in ovarian cancer indicating it could also contribute to increased migration.



(Made with Biorender)

Coculture wound healing assays

Ovcar3 wound healing coculture with MET5A cells to investigate if the presence of Mets promotes faster migration of Ovcar cells. Ovcar3 cells plated alone or + MET5A cells at an equal ratio. Wound heal assay performed and measured with ImageJ.

Figure 1:

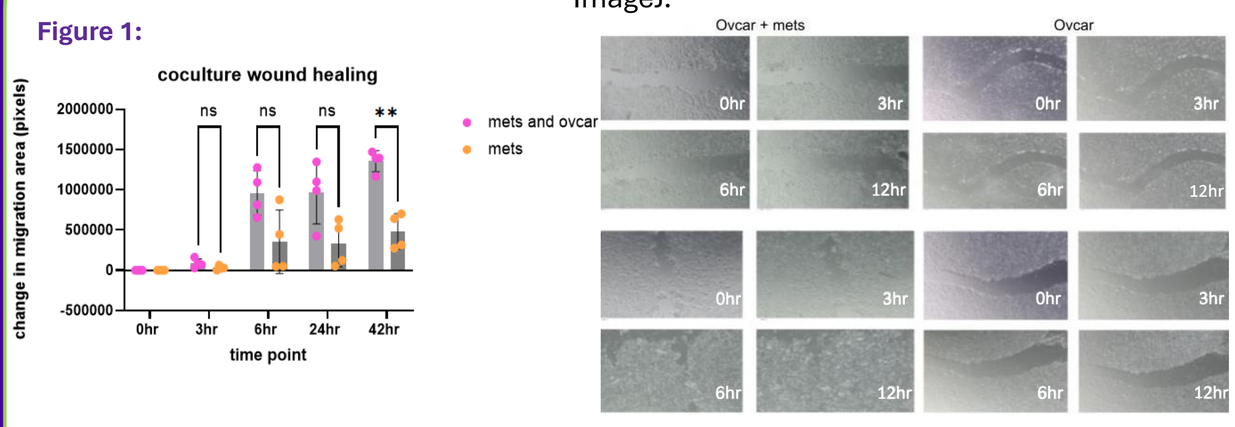


Figure 1: *Two-way anova performed on n=1, * p=0.05 ** p=0.005.*

Ovcar3 wound healing with different condition media to see if secretions from Mets promote migration of Ovcar cells. Media harvested from plates of Ovcar3 and MET5A after 24 hours and applied to Ovcar3 wound heal scratch assay.

Figure 2:

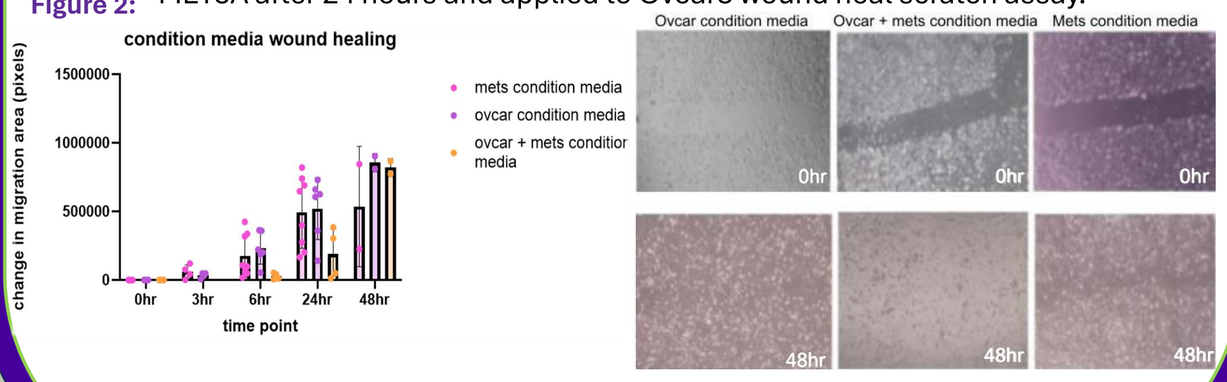


Figure 2: *Two-way anova performed on n=2.*

Fibronectin wound healing assays

Ovcar3 wound healing in different conditions to investigate the role of fibronectin in cancer progression. Matrigel or Matrigel + fibronectin (fn) added to recreate a 3D environment. Wound heal assay was performed, and area measured using ImageJ.

Figure 3:

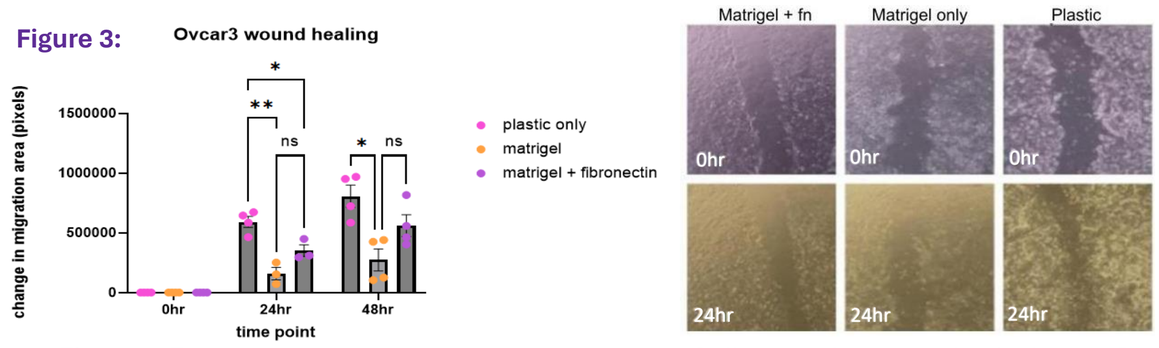


Figure 3: *Two-way anova on n=1, * p=0.05 ** p=0.005. Cells move faster on plastic than a 3D environment , no significance but fibronectin faster than Matrigel visibly. The higher significance between plastic and Matrigel compared to Matrigel + fibronectin at 24hrs suggests the fibronectin increases migration.*

ADAMTS5 role in Ovcar3 migration investigated with ADAMTS5 inhibitor or DMSO. Ovcar3 cells covered by either Matrigel or Matrigel + fibronectin (fn) and DMSO or inhibitor was added. Wound heal assay was performed and area measured with ImageJ.

Figure 4:

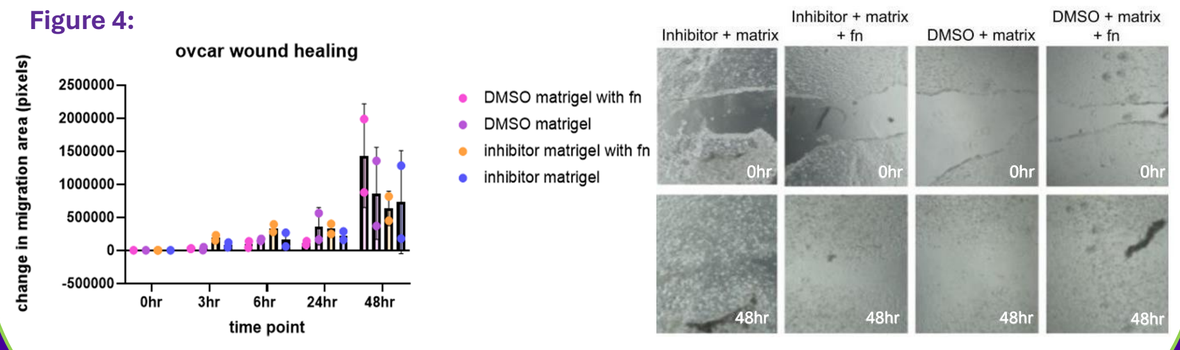


Figure 4: *two-way anova performed on n=1.*

Visualisation of adhesion components

Western blot for Beta1, Alpha2 integrins and fibronectin in Ovcar3 and A2780 human ovarian cancer cell line, fluorescence measured using image studio.

Figure 5:

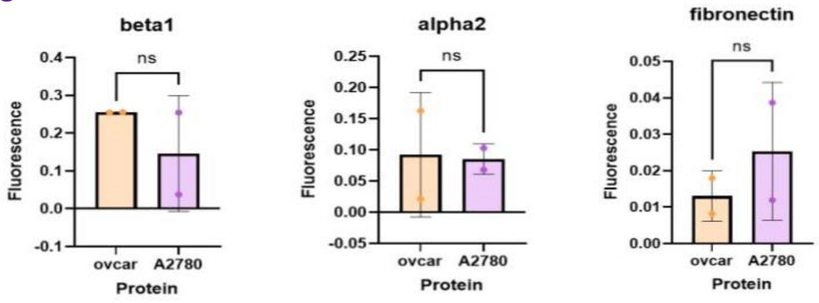


Figure 5: *two-way anova was performed for n=1, no significance due to lack of repeats.*

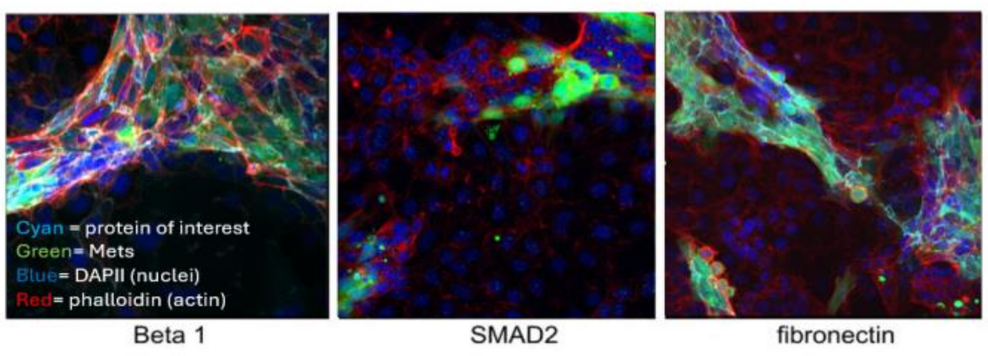
Figure 6: *Antibody staining in coculture*

- *SMAD2 = downstream effector of TGFb a promotor of CAF behaviour*
- *Fibronectin to see if present*
- *B1 integrin= binds fibronectin*

Interestingly beta 1 and fibronectin were not observed around the Ovcar but were observed around the Mets.

Antibody staining on MET5A and Ovcar3 coculture to investigate If Mets were converted to CAF's by Ovcar cells, possibly through secretions.

Figure 6:



Conclusions

- Mets improve migration of Ovcar compared to Ovcar only in a 3D environment as more migration was seen in the same period compared to Ovcar only.
- Cells treated with condition media from Ovcar and Mets did not appear to migrate more quickly at first but then showed a large spike compared to Ovcar condition media which showed stable closure.
- Fibronectin increased migration of Ovcar cells compared to cells plated with Matrigel only.
- ADAMTS5 inhibitor decreased the migration of Ovcar cells.
- Staining for beta 1 and fibronectin present around the Mets but not Ovcar. Mets possibly adhering and producing fibronectin which is used by the Ovcar.

References

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