Effect of Focal Adhesion Kinase Inhibitors on Triple Negative Breast Cancer in the presence and absence of Extracellular Matrix

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1.Overview:

- Extracellular matrix (ECM) surrounds tumourigenic and non-tumourigenic cells and regulates signalling pathways within cells such as focal adhesion kinase (FAK).
- Targeting FAK regulation by ECM may be a potential therapeutic strategy in breast cancer treatment.

2. Introduction:

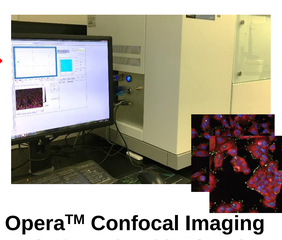
- ECM is a dynamic niche consisting of water, fibrous proteins (eg: Collagen, Laminin, Fibronectin) and proteoglycans intercalated in a complex network and is part of the cell microenvironment which regulates cellular signalling pathways.
- FAK is a non-receptor protein tyrosine kinase involved in cell invasion and migration leading to tumour progression and ECM-cell membrane receptor interactions regulate the activation of FAK.
- The aim of this study was to determine how the specific ECM protein, Collagen IV, impacts FAK activation; and determine the effect the FAK inhibitor Y15, has on FAK, cell cycle arrest and DNA damage leading to apoptosis of the triple negative breast cancer cell line, MDA-MB-231.

3. Methods:

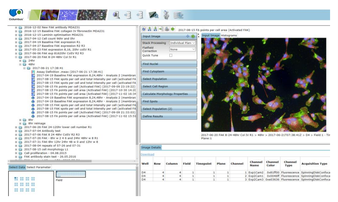
Changes in FAK activation in the presence and absence of Collagen IV: Imaging based assay

384 well CellCarrier plate

FAK inhibitor: Y15



Opera™ Confocal Imaging Platform (PerkinElmer)



Columbus™ Data Management System (PerkinElmer)

Collagen IV (10 – 250µg/mL)
No Collagen IV – control
Negative control: 0.4% DMSO

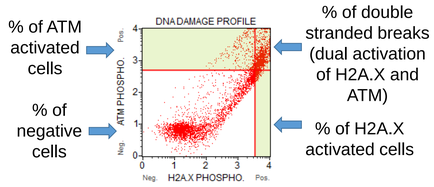
Detection of cell cycle arrest and DNA damage in the presence and absence of Collagen IV following FAK inhibitor addition: MUSE assay (cell sorting)

24 well plate

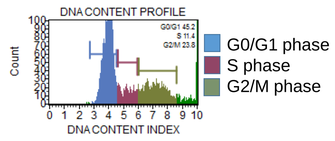
FAK inhibitor: Y15

Sample preparation

MUSE® Cell Analyser (Merck)



DNA damage analysis

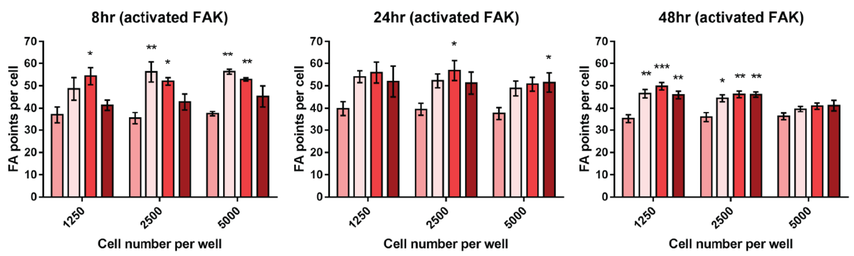


Cell cycle analysis

Collagen IV (100µg/mL)
No Collagen IV – control

Negative control: 0.4% DMSO
Positive control: Etoposide 5µM
(DNA damage assay)

4. Results:



*p < 0.05, **p < 0.01, ***p < 0.001, One-Way ANOVA and Dunnet's multiple comparisons test.

Figure 1: Changes in the number of focal adhesion (FA) points per cell in the absence and presence of Collagen IV at 8, 24 and 48hr. The MDA-MB-231 cells were grown in the absence and presence of Collagen IV at concentrations of 10, 100 and 250 µg/mL. The cells were imaged using the Opera™ Confocal Imaging Platform (PerkinElmer), x20 Objective and the results were analysed using the Columbus™ Data Management Software (PerkinElmer). The graphs represent the mean of two replicates in three independent experiments. Error bars represent the mean ± SEM.

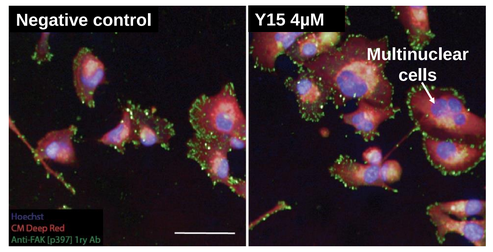
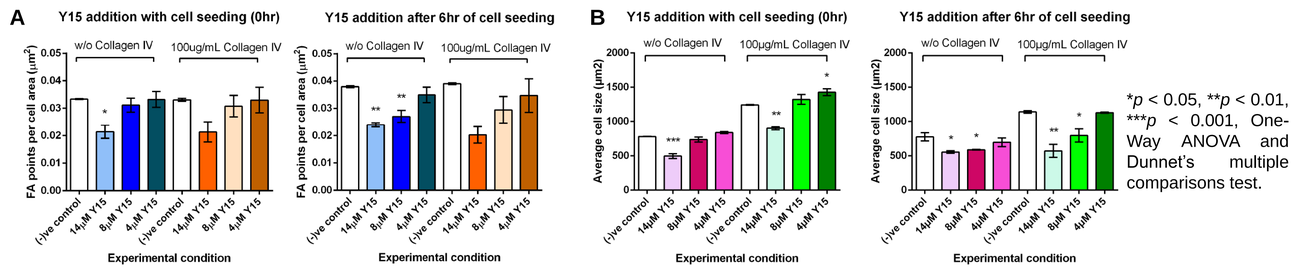


Figure 2: Changes in the MDA-MB-231 cell morphology in the presence of 100ug/mL Collagen IV with and without Y15 at 8hr. The MDA-MB-231 cells were grown in the presence of Collagen IV at 100 µg/mL. The cells were imaged using the Opera™, x20 Objective (Scale bar: 50µm).



*p < 0.05, **p < 0.01, ***p < 0.001, One-Way ANOVA and Dunnet's multiple comparisons test.

Figure 3: Changes in the number of FA points per cell area and cell size in the absence and presence of Collagen IV at 8hr. A: Changes in the number of FA points per cell area **B:** Changes in the average cell size. The MDA-MB-231 cells were grown in the absence and presence of Collagen IV at 100µg/mL. The cells were imaged using the Opera™ and the results were analysed as described above. The graphs represent the mean of two replicates in three independent experiments. Error bars represent the mean ± SEM.

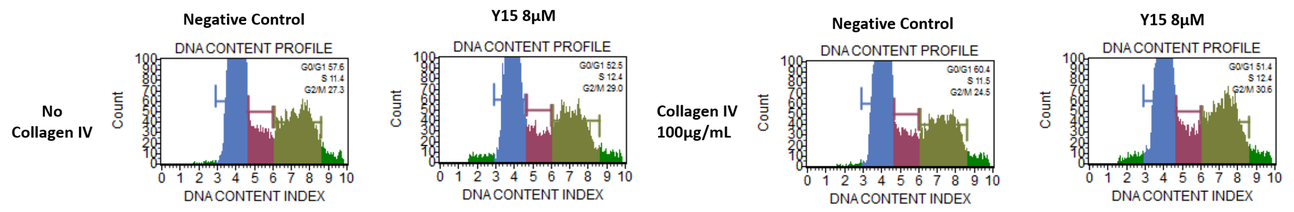
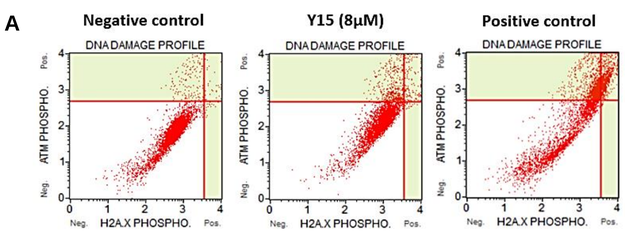


Figure 4: Cell cycle analysis in the absence and presence of Collagen IV following Y15 addition. The MDA-MB-231 cells were grown in the absence and presence of Collagen IV at 100 µg/mL, Y15 was added at 8µM (IC_{50}) and the cells were incubated for 96hr. The cell samples were prepared and analysed using the MUSE® Cell Analyser (Merck). The results are representative of three independent experiments.



B

	w/o Collagen IV			With 100µg/mL Collagen IV		
	Negative control	Y15 (8µM)	Positive control	Negative control	Y15 (8µM)	Positive control
Negative (LL)	96.83 %	83.67 %	57.66 %	96.81 %	82.40 %	59.70 %
pATM (Single Pos) (UL)	2.05 %	12.94 %	23.64 %	2.36 %	13.30 %	31.58 %
DNA double-strand breaks (UR)	0.46 %	3.17 %	17.27 %	0.40 %	3.90 %	8.62 %
pH2A.X (Single Pos) (LR)	0.66 %	0.22 %	1.43 %	0.42 %	0.40 %	0.10 %
Total DNA Damage (UL+UR+LR)	3.17 %	16.33 %	42.34 %	3.19 %	17.60 %	40.30 %

Figure 5: DNA damage analysis in the absence and presence of Collagen IV following Y15 addition. A: DNA damage profiles for negative control, Y15 and positive control **B:** Percentages of cell population which exhibit DNA damage under each experimental condition. The MDA-MB-231 cells were grown in the absence and presence of Collagen IV at 100 µg/mL, Y15 was added at 8µM (IC_{50}) and the cells were incubated for 72hr. The cell samples were prepared and analysed using the MUSE®. The results are representative of two independent experiments.

5. Conclusions and Future Directions:

- A significant increase in FA point formation in MDA-MB-231 cells was observed in the presence of Collagen IV suggesting a role in FAK activation.
- The concentration dependent response observed in FA point formation per cell area in the presence of Y15 indicates that FAK inhibition in MDA-MB-231 cells can be achieved with short incubation periods.
- The increased cell size after Y15 addition, which is more evident in the presence of Collagen IV, was not due to cell cycle alterations. However, Y15 causes DNA damage, irrespective of the presence of Collagen IV, which is an indication of apoptosis.
- The effect of Y15 on MDA-MB-231 will be further investigated in the absence and presence of ECM protein combinations.

ACKNOWLEDGEMENTS: Thilini Perera is supported by an Australian Government Research Training Program Scholarship. Thilini Perera was awarded a Tony B. Academic Travel Award to attend the SLAS 2018 International Conference and Exhibition.