

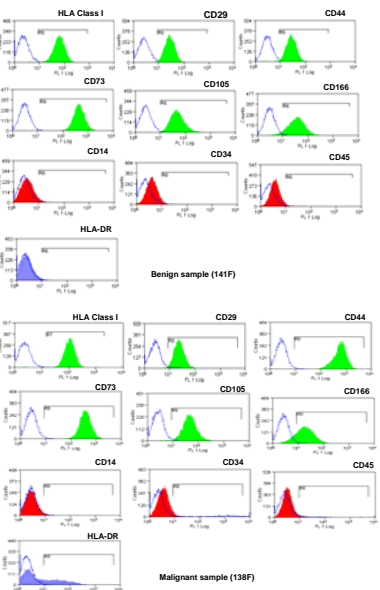
Lim, M.L. Susan^{3,4}, Lam F.L.⁴, Tang, G.C. Kerrie¹, Lim Jiahao Daisy², Tan Bee Tee², Tanavde Vivek², Sakban, Rashidah¹, Karuppasamy, Jayavani¹, Ma F.³ and Lee E.H.¹.
¹ Department of Orthopaedic Surgery, National University of Singapore, Singapore
² Bioinformatics Institute, Agency for Science Technology and Research (A*STAR), Biopolis, Singapore
³ Stem Cell Technologies (i), Singapore.
⁴ Susan Lim Surgery, Centre for Breast Screening & Surgery, Singapore Email: sl@susanlimsurgery.com

Mesenchymal stem cells (MSCs) are multipotent adult stem cells found in many tissues in the human body including the bone marrow and adipose tissue. These cells can also be isolated from the stroma of benign and malignant breast tissue.

The aim of this study is to isolate and perform a comparison of the immunophenotype and gene expression profiles of MSCs in the stroma of both benign and malignant breast tissue, and to determine if these differences may contribute to our understanding of the interaction of stromal MSCs with cancer cells in the tissue-specific microenvironment, and to cancer progression and metastases.

Methods

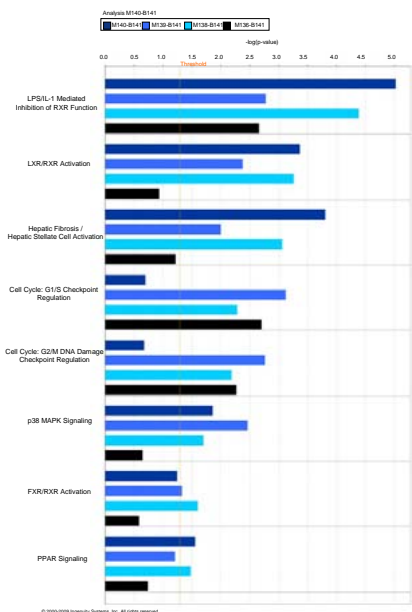
MSCs in culture, between passages 1 to 6, derived from 4 patient samples of benign and malignant breast tissue each, were used for all experiments. Phenotypic characterization was performed by flow cytometry with a panel of fluorescent-labelled specific antibodies. The gene expression profiles were compared using Human Ref 8 V3 arrays (Illumina Inc., CA). The data was analyzed using Genespring (Agilent Inc., Singapore) and analysis of signaling pathways was conducted using the Ingenuity Pathways Analysis platform (Ingenuity Inc., CA).



Antigen	Benign samples (n=4)	Malignant samples (n=4)
HLA Class I	97.4 ± 0.5%	97.2 ± 0.8%
CD29	88.0 ± 11.7%	88.1 ± 12.2%
CD44	97.7 ± 0.7%	97.2 ± 0.8%
CD73	98.3 ± 0.3%	97.5 ± 1.0%
CD105	94.8 ± 5.1%	93.8 ± 4.8%
CD166	87.9 ± 10.2%	90.6 ± 6.4%
CD14	1.6 ± 0.7%	1.2 ± 1.3%
CD34	1.7 ± 0.7%	1.7 ± 1.9%
CD45	0.7 ± 0.8%	0.4 ± 0.2%
HLA-DR	1.3 ± 1.0%	19.3 ± 22.9%

Table 1: Table showing the percentages of cells expressing the surface antigen, as determined by flow cytometric analyses. Values are expressed as mean ± SD.

Figure 1: Flow cytometric analysis of the surface phenotype of cultured adipose stromal cells. A representative profile is shown for each benign and malignant sample. Solid histograms are with antigen specific antibodies and open histograms are isotype controls.



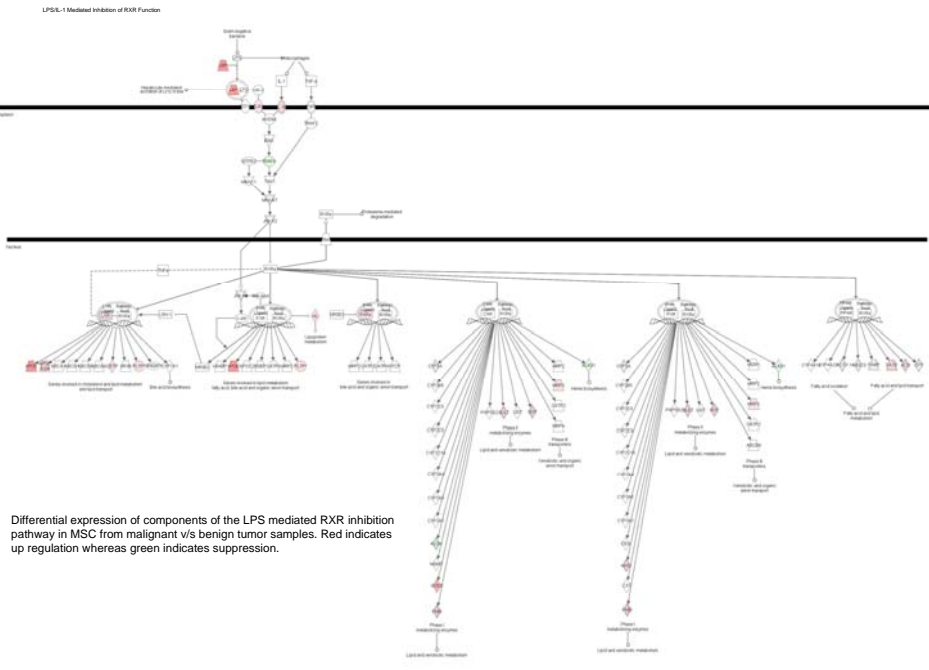
Differentially expressed signaling pathways in adipose MSCs from malignant v/s benign tumors

Results

Flow cytometric analyses revealed that the immunophenotype of MSCs from breast stroma in both benign and malignant groups, were positive for HLA class I, CD29, CD44, CD73, CD90, CD105, and CD166, and negative for the hematopoietic lineage markers CD14, CD34 and CD45. Additionally, significant percentages of HLA-DR positive MSCs were found in samples belonging to the malignant group (Figure 1 and Table 1).

An analysis of gene expression profiles showed that on average, 1000 genes were differentially expressed in MSCs in the malignant group, compared to the benign group. Of these, 190 genes were differentially expressed across all MSC samples from the malignant group.

The differentially expressed genes were grouped by cell function and diseases using the Ingenuity Pathways Analysis. It was found that genes involved in cellular movement, cell to cell signaling and interaction, cardiovascular system development, lipid metabolism, cancer, respiratory disease, cardiovascular disease, renal disease and inflammatory disease were upregulated in MSCs from the malignant group. Genes involved in LPS-mediated inhibition of RXR function, FXR/RXR activation, PXR/RXR activation and LXR/RXR activation were also significantly over expressed in MSCs from the malignant group.



Differential expression of components of the LPS mediated RXR inhibition pathway in MSC from malignant v/s benign tumor samples. Red indicates up regulation whereas green indicates suppression.

Conclusion

From this data, we conclude that the phenotype of breast stromal MSCs is similar to that originally described for MSCs (Caplan, 1991) but negative for hematopoietic lineage markers. Additionally, MSCs derived from malignant breast tissue were found to significantly express HLA-DR, which warrants further investigation. Distinct gene expression profiles were also found for MSCs from malignant breast tissues, compared to benign samples. Bioinformatics analysis of gene function and signaling pathways involved predicts that MSCs from malignant breast samples may have a significant effect on the cancer microenvironment through the activation of genes involved in various cellular functions and via lipid activated nuclear receptors like the LXR and RXR receptors. Further work in this area may contribute to our understanding of the role of breast stromal MSCs in regulating invasion and metastasis of breast cancer cells.

Acknowledgement

The authors wish to acknowledge Dr Remulla Sherwin Jade, Mr Jonathan CL Koh and Ms Christine GY Neoh for their assistance in the production of the poster.