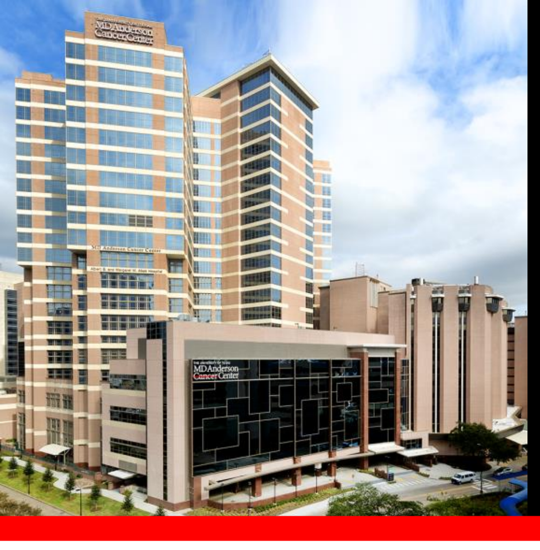

Lymphatic Endothelial Cell Secretion Effect On Inflammatory Breast Cancer Cell Growth

Kara Smeltzer[1], Wintana Balema[2], Jessica Li[2], Richard Larson[2], Wendy A. Woodward[2]

Baylor University[1], MD Anderson Cancer Center[2]

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

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Background

- Inflammatory breast cancer (IBC) is rare representing 2-4% of breast cancers[1]
- IBC is highly life-threatening representing 7-10% of breast cancer-related deaths in Western countries[2]
- IBC cases involve dermal-lymphatic invasion (DLI) which is related to rapid metastasis[3]
- It is unknown if lymphatic endothelial cells (LECs) induce DLI
- Lipocalin-2 is upregulated in IBC tumor cells and is described as a lymphatic regulator
- We hypothesize that conditioned media (CM) from LECs will increase IBC cell growth in a lipocalin-2 (LCN2) dependent manner

Methods

Colony Assay 1: IBC3 Cells +/- LEC CM

1. Culture IBC3 cells, LECs, IBC3 shLCN2 cells
2. Remove media from LECs after 48 hours
3. Seed IBC3 cells; experimental have 25% LEC CM; control have IBC3 media
4. Fix cells with methanol
5. Stain with crystal violet
6. Count colonies using Fiji software

Colony Assay 2: IBC3 Control and shLCN2 Cells +/- LEC CM +/- RT

1. Culture Control and shLCN2 IBC3 cells and LECs
2. Remove media from LECs after 48 hours
3. Seed IBC3 cells; experimental have 10% LEC CM; control have IBC3 media
4. Seed IBC3 shLCN2 cells; experimental have 10% LEC CM; control have IBC3 media
5. Irradiate (0, 2, 4, 6 Gy)
6. Fix cells with methanol
7. Stain with crystal violet
8. Count colonies using Fiji software

Results

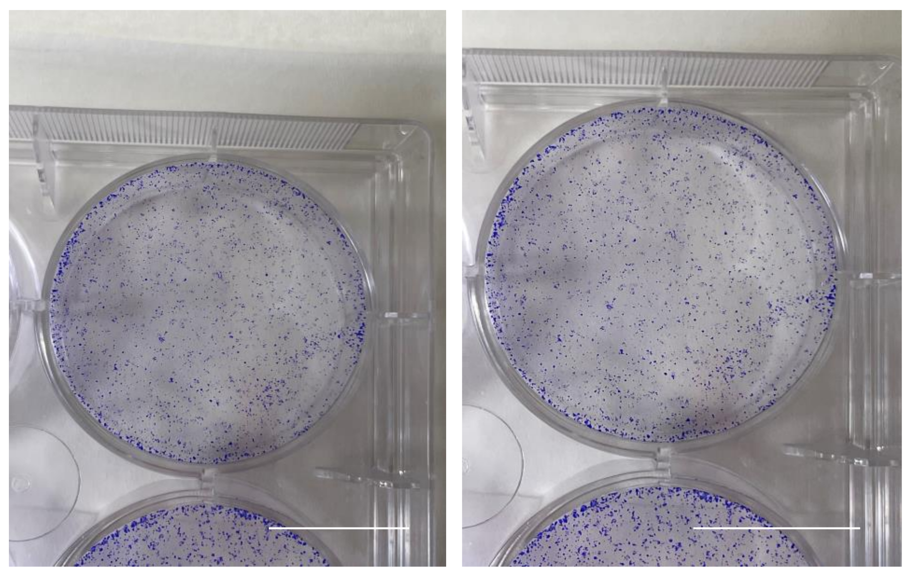

Fig. 1 Colony Photos. Two photographs were taken of each well using a 12MP resolution camera with a 26mm f/1.6 lens. Photo 2 used greater magnification and captured a clearer image. Photo 1 and Photo 2 of Control Well 1 are shown. Scale Bar = 17.5 mm

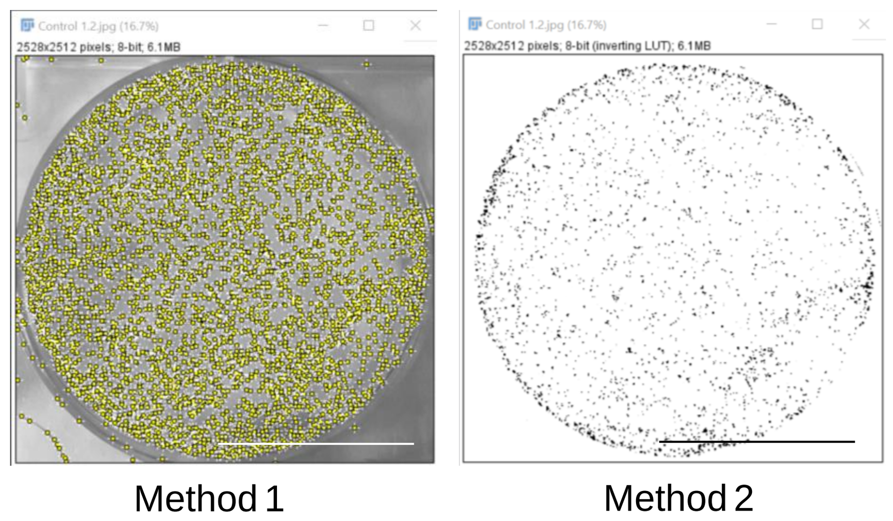

Fig. 2 Photo Analysis. Two methods were used to count the colonies in Fiji, ImageJ. Method 1 used the "Find Maxima" function with prominence set to greater than 20. Method 2 used the "Analyze Particles" function with the threshold set to 80. Control Well 1 is shown under conditions for both methods on Photo 2. Scale Bar = 17.5 mm

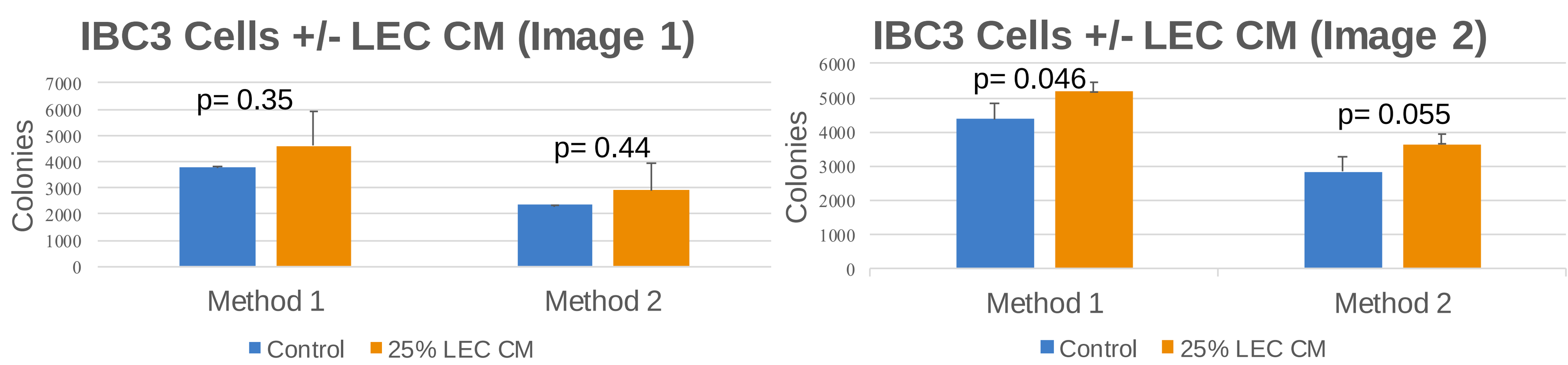

Fig. 3 LEC CM increases IBC3 Colonies. 3A) Average number of colonies in control wells versus experimental wells using both Method 1 and Method 2 on Photo 1. 3B) Average number of colonies in control wells versus experimental wells using both Method 1 and Method 2 on Photo 2. Differences in Photo 2 are significant using Method 1 and trend towards significance using Method 2.

Conclusion

- Adding 25% LEC CM to IBC3 cells increases colony formation compared to control, consistent with our hypothesis.
- Photo 2 was more magnified and counted more colonies. Photo 2 is more representative of the data than Photo 1. When analyzing data gathered from Photo 2, the differences between the control wells and the experimental wells are significant using both counting methods.

Next Steps

- Repeat Colony Assay 1
- Fix, stain, and analyze Colony Assay 2
- Analyze the LEC CM for LCN2
- Run experiment with IBC3 LCN knockout cells with 3 control wells and 3 experimental wells with 25% LEC CM
- Directly test effect of LCN2 on IBC3 cells

References

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