The EPR Effect in Breast Cancer

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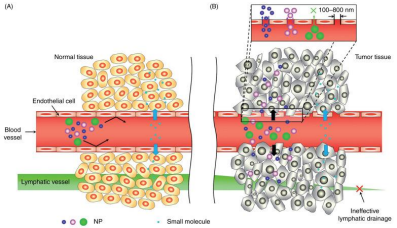
Introduction

The enhanced permeability and retention (EPR) effect was first observed by scientists Hiroshi Maeda and Yasuhiro Matsumura in 1986. The EPR effect is the mechanism by which higher-weight molecules tend to accumulate more in cancerous tissues than normal tissues due to the leaky nature of tumor blood vessels and poorer lymphatic drainage. This effect occurs because macromolecules larger than 40 kDa selectively leak out from tumor vessels and accumulate in tumor tissues.



However, the EPR effect is quite heterogeneous—various factors, such as tumor growth environment and the nature of the vasculature, can impact the strength of the EPR effect. Hiroshi Maeda, who first observed the EPR effect, stated in his paper that pancreatic and prostate cancers tend to have lower vascular densities, so they show poor EPR effect. However, hepatocellular carcinoma and renal carcinoma tend to have high vascular density, so they show greater EPR effect. Despite this association between high vascular density and high EPR strength, breast cancer is highly vascularized but still demonstrates poor EPR effect. This phenomenon in breast cancer suggests that the reason for the weak EPR effect could be because of lymphatic activity instead of blood vessel activity. Moreover, ⅔ of breast cancer patients have lymph node metastasis.

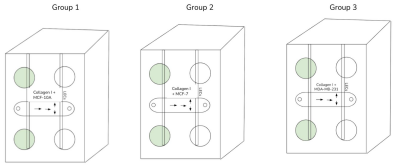
This study hypothesizes that the reason for the EPR effect being weaker in breast cancer is due to increase in lymphatic drainage. This hypothesis was studied by using biomimetic chips with either no breast cancer cells seeded in them, or with breast cancer cells treated in them, to examine if there was an increase in lymphatic drainage.

Method

This experiment utilized MDA-MB-231 cells, a type of triple-negative, highly metastatic human breast cancer, MCF-7, a mild breast cancer, MCF-10A, normal breast tissue, as well as hMVEC-d cells, which are human lymph endothelial cells sourced dermally. These cells were cultured using their standard culturing protocol, which included media changes every three days, as well as passaging about once or twice a week. Once the cells reached P6-8, they were ready to be seeded into the chips.

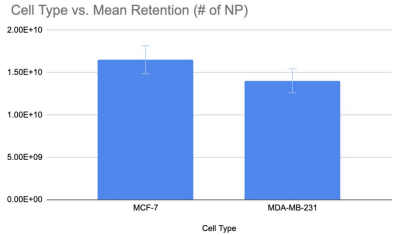
The chips used in this experiment were two-channel chips made out of PDMS. They were created by pouring PDMS and a curing agent into the Lee Lab's moulds, and baking them overnight at 80°C.

Current experimental data only compares MDA-MB-231 and MCF-7 cells, but the next step is to also compare MCF-10A cells. Thus, chips were separated into two groups, treated with a collagen and cell mixture. The next day, a green nanoparticle solution with concentration 2.46E+08 nanoparticles/uL was created, and 250 uL of this solution were added to the two left media reservoirs of the chips. The chips were then left on the rocker for 16 hours.



The following day, the drainage was measured from the left side of the chip to the right. This was done so by measuring the mass of the solution on the right side. The density of the nanoparticle solution, 1.05E-6 g/uL was then divided by this number to get the resulting volume. This volume was then multiplied by the concentration, which was measured using a spectrophotometer, to get the number of nanoparticles on either side of the chip. Then, the number of nanoparticles on the left side of the chip was subtracted from the number on the right, resulting in the number of nanoparticles in the bulk, which represented the "retention" in the corresponding cell type.

Results



The above graph shows total nanoparticle drainage versus cell type. This data was across 13 chips with a p-value of 0.03552097047.

As demonstrated by the p-value above, there is a somewhat statistically significant difference between the retention in the MCF-7 versus MDA-MB-231 cells, which is consistent with our hypothesis that there is weaker EPR effect in MDA-MB-231 cells.. This means that there are more nanoparticles in the bulk of the MCF-7 group versus the group with MDA-MB-231. The biomimetic chip's bulk represents the nanoparticles that have been uptook but not drained by the lymph endothelial cells. Therefore, a greater number of nanoparticles in the bulk means that there was more retention, which supports the original hypothesis that there would be less retention and the lymph vessels would be hyperactive.

The next step in this experiment is to compare this data against MCF-10A cells, which are normal breast tissue cells. Moreover, the junctions in the lymphatic vessels should be imaged to see differences between the different cell types qualitatively.

Conclusion

Ultimately, the results of this experiment support the original hypothesis—the results suggest that there was more retention in severe breast cancer compared to more mild cancer. However, the significance of the result was not as much as expected. Thus, this experiment should be repeated in the future using normal breast tissue as a true control. Finding the reason for poor EPR effect in breast cancer could lead to innovations that can increase EPR effect strength. This would be a major breakthrough in passive targeting strategies for drug delivery, and help reduce negative effects of cancer therapy on normal cells.

Acknowledgements

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