Examining the role of exosomes in the spread of chondrosarcoma

| Zoe Bell, 160054347, z.bell@newcastle.ac.uk |Physics MPhys Honours | School of Maths, Stats and Physics |
| Supervisor: Dr Daniel Frankel |

Newcastle University
Northern Institute for Cancer Research

Aims

- To grow chondrosarcoma cells and isolate exosomes from these cells
- Identify the isolated exosomes using dynamic light scattering
- Observe how cells move in response to exosomes using an impedance based migration chamber

Introduction

According to Cancer Research UK, approximately 350 people die each year to bone sarcomas which indicates about 1 person dies due to a bone sarcoma every day in the UK[1].

Chondrosarcoma is a type of bone cancer which grows inside a bone or on its surface. Chondrosarcoma affects the cartilage which is the connective tissue that connects bones to joints. The cancer can sometimes metastasize (spread) to other parts of the body such as nearby tissues or organs like the lungs[2].

Exosomes are extracellular vesicles which are secreted in high numbers by cancer cells. Increasing evidence suggests that the exosomes released by cancer cells can alter the microenvironment locally and at distant sites. This allows the cancer to metastasize because the exosomes ensure that the new environment is ideal for the cancerous cells to grow[3].

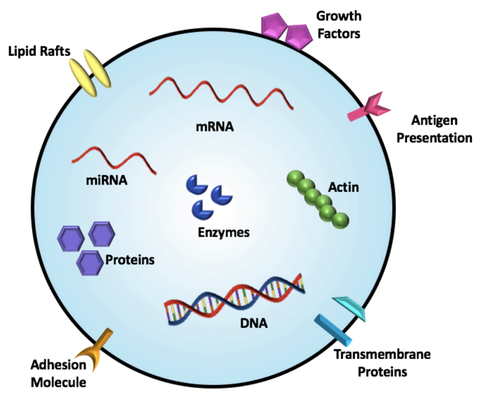

Figure 1: Diagram of an exosome showing some of the molecules that it contains.

Method

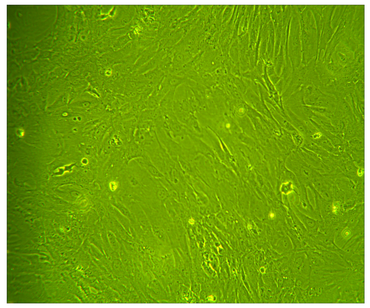

Figure 2: Microscope image of the SW 1353 chondrosarcoma cells.

The SW 1353 chondrosarcoma cells were cultured in a medium of RPMI-1640 and stored in an incubator at 37°C. Exosomes were isolated from the cell media using an exosome isolation kit followed by centrifugation. Light scattering was used to measure the size of the isolated particles in solution in order to confirm the characteristic size of exosomes.

Cells were placed in a serum free medium (without nutrients) in the top half of an invasion chamber with the exosomes placed in serum containing medium at the bottom of the chamber. As cells pass through the microporous membrane they alter the impedance of gold electrodes, thus allowing cell migration to be recorded. Impedance was measured for 24 hours to follow cell migration. In some experiments an extracellular matrix mimic, Matrigel, was added to more closely replicate the tumour environment in vivo.

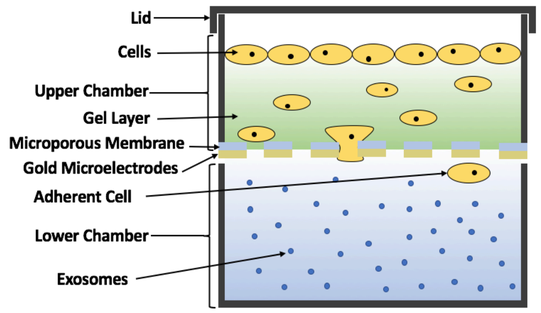

Figure 3: A single well of the cell invasion assay plate.

Results and Discussion

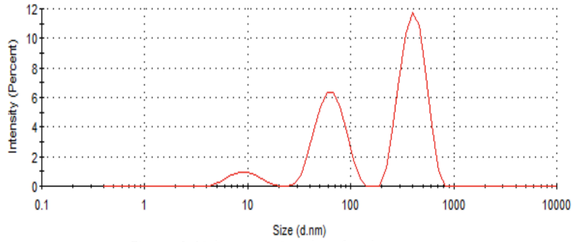

Figure 4: Light scattering data for the exosomes

Figure 4 shows the data obtained from dynamic light scattering of the exosomes which was performed in order to confirm the presence of exosomes. The first peak is debris, the third peak is possibly aggregated exosomes and the second peak can be assigned to exosomes. The exosomes are about 60 nm in diameter.

Figure 5 shows the migration data of cells through the chamber. The cell index is a measure of impedance which increases as a cell passes through the microporous membrane. Cells from the upper chamber move towards the lower chamber because this contains nutrients and chemical signals.

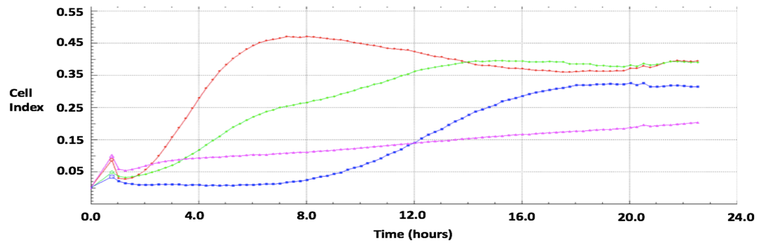

Figure 5: Cell index over time for the cells migrating through the chamber. The red, blue and green curves represent cells invading through the chamber with Matrigel and the purple curve represents cells invading without Matrigel.

The purple curve (without Matrigel) shows that cells move to the other side of the membrane towards the nutrients/exosomes as the cell index increases with time.

The red, blue and green curves (with Matrigel) have steep sections, which represent an invasion phase where cells invade through the gel using enzymes to break down the extracellular matrix (the area supporting cells). This suggests that exosomes encourage cells to move towards them through tissues of the body.

Conclusions

- Exosomes were successfully isolated from chondrosarcoma cells.
- The diameter of the exosomes was about 60 nm.
- Cells in the invasion assay chamber moved towards the exosomes/nutrients and, invaded through the matrix to do this.
- Further work could include using microfluidic channels to observe how exosomes interact with cells in the blood.

Acknowledgements

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