|  |  |  |  |
| --- | --- | --- | --- |
| GenomeFolder | Contaminants | RNA | miRNA |
| Homo\_sapiens\UCSC\hg19\Sequence\WholeGenomeFASTA | HumanRNAContaminants | hairpin.fa | mature.fa |
| Homo\_sapiens\UCSC\hg19\Sequence\WholeGenomeFASTA | HumanRNAContaminants | hairpin.fa | mature.fa |
| Homo\_sapiens\UCSC\hg19\Sequence\WholeGenomeFASTA | HumanRNAContaminants | hairpin.fa | mature.fa |
| Homo\_sapiens\UCSC\hg19\Sequence\WholeGenomeFASTA | HumanRNAContaminants | hairpin.fa | mature.fa |

Introduction:

* Prostate cancer: second leading cancer related death.
* Primary tumours have 5 five year survival, however metastasis is reduced to only 12-15months.
* Metastasis appears to be triggered by an induction of the tumour microenvironment.
  + This include cancer associated fibroblasts
  + Tumour cells
  + Immune cells
  + Adipocytes and etc.
  + Function by cell-cell interaction and paracrine interaction.
  + This interaction can be facilitated by exosomes.
* Exosomes; little known about their formation, sorting and content under specific conditions.
* Contain mRNA, microRNAs, proteins and in some cases, DNA.
* Strong links to metastasis have been made by inhibiting its production in cancer cells that exert metastasis.
* Adding exosomes to naïve cells has previously been found to induce certain behaviours depending on their content
* Hypoxia: Induced due to a lack of vasculature in the growing tumour.
* Known to induce proteomic and genetic changes in attempt to prolong its own survival.
* Seems to correlate with metastasis, however its away from the invasive front so this method of metastasis is likely to be in the form of cell-cell communication. IE, exosomes.
* However the content that causes this is unknown.
* Hypothesis; That hypoxic prostate tumour cells secrete exosomes containing pro-invasive, and metastasising proteins which can be absorbed by surrounding cells.
* Aims:
* Overarching methods:
  + Used both LNcap and PC3, different only due to androgen sensitivity. Need to explain this.
* Results and methods:

1; characterisation of LNcap EXOnorm and EXOhyp: Here they attempted to figure out if the overarching exosomes are different.

Method: grew LNcap at normal conditions and a sample at hypoxic conditions known as 1%. From here they extracted the vesicles using a standard method using ultracentrifugation at high speed. ISSUE WITH SPEED?

Samples were then put into a nanoparticle tracking machine which works off the basis of Brownian motion, where size of particles affects the amount of Brownian motion that is exerted.

This reveals a size disruption between the two cell types, where the total amount of exosomes secreted were similar. However, by analysing the cells in total, the hypoxic cells grow slower obviously due to the uncomfortable conditions. DISCUSSION NEEDED.

Following this they looked at the actual content of the exosomes. Firstly, they looked for typical exosome markers. WHY? Used equal protein concentrations to do this, but perhaps the ratio of what’s in there could be different?

2; EXOhypoxic enhance invasiveness and motility of PCA cells.

Invasiveness: used LNCaP norm and hypoxic cell exosomes and applied them to naïve LNCap cells. The setup is a little weird, where there is one membrane located on top of a lower chamber. Applied exosomes to the cells, and then were seeded in the upper chamber. If cells aere invasive they move throught the membrane intothe lower chamber. This is what was shown in figure 2, where this is quantified in this graph. Pretty self-explanatory and had resulted in a large difference. Not sure about what they quantified though.

Secondly, they tested migration; Basic procedure we would have all had to have done in undergrad. Here they used PC3 cells, grown to 100% then scratched down. Initial scratch recorded and then exosomes were added. Another image was taken 6hrs post stimuli.

Varying levels of initial confluencies? Control? Diffence between normoxic and control non-existant. However hypoxic to control/normoxic is very obvious with an increase of approximately 50% of the normal oxygen cells.

This is enough to believe that the exosomes are indeed harbouring something pro invasive and metastasic, however what this is is still unknown.