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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. http://search.proquest.com.ezproxy.library.uq.edu.au/docview/1030083674/abstract/602CF780E8824E9FPQ/1?accountid=14723
* 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 metastatic, however what this is is still unknown.

3: EXOhyp promote stemness in PCA cells and promote CAF-phenotype. Here they assessed stemness, which is effectively the word to describe something being stem-cell like. This means they are less adherent, possess the ability to differentiate etc. To measure this occurrence, they measure the formation of prostaspheres, which are essentially spherical clumps of cells from the prostate progenitor due to limited adherence. Here they counted (by eye?) the spheroid formation and observed quite a large difference between normoxic and hypoxic cell types. Again they didn’t specify the controls.

Fibroblasts are cells that are typically responsible for tissue connectivity. Now fibroblasts can be stimulated in inflammatory conditions to allow for would healing. However, in cancerous conditions, the effected cells can secrete cytokines and other factors that also promotes this response in the fibroblasts, resulting in what is known as the cancer associated fibroblasts (CAFs). In the mid-2000s, multiple expression studies was performed to determine what it is about these cells that allow for their role in cancer; typically they express vascular endothelial-derided growth factor and several other growth factors, as well as secrete a number of matrix-remodelling enzymes such as MMPs. Additionally, their own morphology is changed, which can be detected as an increase in alpha-smooth muscle actin (alpha-SMA), commonly used as a marker to assess for CAFs in tumour tissues as it has been linked to an increase in tumour growth, metastasis and angiogenesis.

Here they assessed the amount of actin in fibroblasts (PrSC) when exposed to no exosomes, normoxic exosomes, hypoxic exosomes and a positive control of TGF-beta. TGF is known to induce stemness of the fibroblasts and is expected to increase the actin amount within, hence positive control. Representative images definitely show a distinct shift in fluorescence corresponding to increasing actin filaments, where the hypoxic conditions show similar actin amounts to the positive control. Now the quantification uses discrete values between 0-4 where 0 indicates no colour and 4 is bright colouration. This was averaged across 20 different images, I’m assuming 5 images per condition? Here we see a significant increase of the actin expression compared to normoxic, where normoxic was greater than basal. Also, the positive control is greater than the hypoxic, which may indicate an increase in CAF phenotype, however isn’t as strong as the typical reaction.

So now we know what is affected, eg migration, invasion, increased in stemness and enhancement of CAF-phenotype strongly associated with metastasis being affected directly from the exosomal content, however, we still don’t know how.

4: EXOhyp exhibit enhanced MMPs and signalling activity.

First they assessed MMP activity. Here they used a gelatin zymograph gel, which is supposed to be incredibly sensitive for MMP-2 and 9 specifically. They cannot run the MMP on a normal gel due to the activation and deactivation that occurs to these proteins. Without the conditions specific to the zymograph, the MMPs can degrade the gel and destroy the results. Here, we do find a difference in concentration of the MMP2 and 9 in the hypoxic exosomes. Similarly, there is a general increase in signalling molecules listed, which all seem to be associated with general metastasis. Buuut, this particular analysis is not very objective, where only a select few proteins were considered. Hereby a more comprehensive assessment was required.

5: Characterization of exosomal proteins by mass spec.

So, here they performed a high throughput assessment of the exosomal content by mass spectrometry. First they looked at broad similarities and differences between cell types. The supplementary figure provides the full list of proteins in each. Not really important except that it shows that these are indeed very different samples of the cellular environment. Following this, an assessment of their pathway targets was completed using the Ingenuity software, particularly the ingenuity pathway analysis. EXPLAIN. Here you can see enrichment in several different pathways, particularly those relating to adherens and cytoskeletal structure, with some additional ones in cellular signalling.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3973916/

Overall results and conclusion: The hypoxic cells secrete a differential amount and type of proteins.

* These proteins target adheren junctions.
* This is turn results in increased stemness, invasiveness and migration.

They proposed a model where the MMPs from the exosomes is able to degrade the cadherins, releasing the catenins which triggers a pro-metastatic pathway. DISCUSS FIGURE 6.

Now question the proposed mechanism they discussed: information surrounding MMP2 degrading cadherin. Is it on the outer surface or is it inside? Also, other mechanisms, such as the Akt or the exosomal catenin which can be absorbed directly from the exosome.

Future directions:

* Never assessed that it seems that the exosome biogenesis/release was doubled. This may be a point of interest when it comes to therapies.
* Alsoo, the other pathways should be assessed for a comprehensive assessment for the role of these exosomes in cancer progression.
* This only looks at protein content. However resent evidence indicates that these vesicles can also contain miRNAs, RNAs and in some cases there has been reports of DNA transfers intracellularly through exosomes which could be inducing these changes.
* Other vesicle populations. It would be nice to know what population is affected as it reveals some fundamentals of the cellular environment. Eg lipid composition, stresses.

Strengths:

* Determined the subpopulation that is the causative vesicle.
* Use of several cell lines, both cancerous and healthy.
* Published the proteins in exosome content.
* Great starting point; multiple leads are now present.

Limitations:

* Some clarity is lacking. Eg no disclosure of the controls in some experiments.
* Premature conclusions. (adherens targeted based on correlation)
* Absorption of these exosomes affect other cell types.