Slide 1: Title

Slide 2: Exosomes:

- Lesser known cellular component: exosomes.

- A class of a larger family called extracellular vesicles. Primarily composed of microvesicles and exoosmes, but there are several other subpopulation.

- Previously, exosomes weren’t too interesting due to being considered as a garbage disposal.. However, upon finding that the biological contents these vesicles harbor, such as RNA, micrornas proteins etc, was able to be absorbed into recipient cells .. Solidifying a role in intercellular communications.

- Information regarding biogenesis, sorting and the conditions which modify the content are still being elucidated. This is somewhat alarming considering the plethora of evidence coming forward regarding the ubiquitous function of these little vesicles.

-For instance, recent work seems to link exosome release to normal biological functions such as maintaining homeostasis, stress responses and immunity. Whilst also linking exosomes to a role in cancer progression.

Slide3: Exosomes in cancer

* In particular cancer derived exosomes are becoming a hot topic at the moment.
* While most mechanisms and details of what is occurring is still unknown, knockdown and exposure studies using cancer derived exosomes proves very interesting, in that inhibiting exosome release reduces aggressive behavior, and exposure to these exosomes induces, similar to what is found this this paper.
* However, some hypotheses suggest that this may be due to the exosome content educating the cells in pre-metastasis niches or tumour microenvironment, thus allowing for an increase in aggressive behavior.
* Because of this information, exosomes are now, and were at the time of this paper, considered a potential therapeutic target and also possess a potential for cancer diagnostics.
* This diagnostics is now a reality. However at the time of publication this would have still been in early stages. However now they are able to use exosomes to detect some biochemistry relating to advanced cancers

Slide 4: Hypoxia in cancer:

* Advanced cancers develop complex microenvironments, which contain immune cells, fibroblasts, and vasculature.
* However as the tumour grows, some cells may no longer be in proximity to adequate oxygen sources and develop hypoxic responses.
* Typically this results in a large transcriptome changes in order to alter metabolism and growth rate in an attempt to sustain survival.
* Unfortunately this can evoke aggressive behaviors in the surrounding cells, such as an increase in invasive behavior. Yet, how this occurs is unknown and quite puzzling, mainly because hypoxic cells are not in proximity to the invasive front that actually exerts this behavior.
* Thus this indicates a distant intercellular communication type mechanism occurring.

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-This is what the authors of this paper hypothesized. More specifically they’ve hypnotized that exosomes from hypoxic prostate cancer cells can induce pro invasive, pro migratory, pro tumorigenic effects in surrounding cells.

- Ive interpreted three distinct aims, as the authors did specify, which the main focus of this paper are.

-Basically, using prostate cancer cellines, LNCaP and PC3 cell lines, they’ve attempted to establish differences in exosomal content between hypoxic and non-hypoxic cells.

-followed by some functional assessment and lastly an attempt to determine causative agent.

Slide6: Differerence in exosome population

* While this might not seem to germane to the central hypothesis, establishing which vesicle subpopulation is actually being looked at is very important in the extracellular vesicle research.
* Different subpopulation are formed in different subcellular components, eg microvesiccles at the plasma membrane whereas the exosomes are formed closer to the center where they may be different material, and therefore they elicit differing reactions.
* This was tested in two different ways: by assessing the size disruption via nanotracker analysis and by looking for typical vesicle markers.
* Nanotracker analysis work by measuring the Brownian motion of particles through fluid, where the amount of movement is proportional to size. Now the different extracellular vesicle subpopulations are somewhat characterized by size.
* Here the authors said that exosomes are smaller than 100nm, however others define it as smaller than 150nm, where microvesicles are 100 to 1000nm. Here we can see a shift between exosome size between the different conditions.
* This is verified by looking at the vesicle markers. CD81, 63, HSP 70, 90 and Annexin are classical markers of exosomes, whereas calnexin is an endoplasma rectiulim marker which can also be found on endosomes. Effectively this determines whether the vesicles collects are actually EVs.

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* Next the authors looked at phenotypes induced by these exosomes.
* SO firstly, an invasion assay was completed using this set up. EXPLAIN
* Altimately this reveals that the hypoxic exosomes are inducing invasive phenotypes.

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