Cancer & Metabolism: Pathways to the future

September 19-21, 2010

Edinburgh Scotland

Organisers: Eyal Gottlieb, Karen Vousden and Abcam
Abstract

Cross-talk between autophagy and apoptosis: An investigation into the signalling mechanisms

Cervical cancer caused by the human papilloma virus (HPV) is the second most commonly diagnosed cancer world-wide. Cervical cancer cells infected with the HPV virus are highly resistant to undergoing apoptosis when treated with cisplatin compared to cervical cancer cells not infected with the virus. Additionally, in response to chemotherapy, cancer cells can utilize and autophagic response as a survival mechanism when exposed to stressful intracellular and extracellular conditions. Pro-autophagic and anti-autophagic treatment has been indicated in cancer treatment with the goal of overcoming resistance to pro-apoptotic chemotherapy, but cross-talk between autophagy and apoptosis is still not fully understood. We therefore aim to exploit this cross-talk between the pathways as a means of sensitising cancer cells to undergo apoptosis at an earlier time point. HeLa cells (cervical cancer cells) were treated with 5 uM cisplatin for 4 and 6h. LC3-II and cleavage of caspase-3 was quantified through Western blotting. Mitochondrial membrane potential and presence of acidic vacuolar compartments were assessed using flow cytometry. Transmission electron microscopy (TEM) was additionally employed to visualise an autophagic/apoptotic response after treatment. It was observed that following treatment with cisplatin, LC3-II accumulation precedes caspase-3 cleavage and thus, apoptosis. These results were reinforced through flow cytometry. Results obtained indicate that autophagy maintains a favourable intracellular metabolic environment capable of sustaining mitochondrial membrane potential for several hours until apoptosis is induced. Correlation with an in vivo scenario is recommended for future studies.
**September 19, 2010**

Talks of interest and of relevance to my research work:

**Lewis Cantley (Beth Israel Deaconess Medical Center, US)**

“PI 3 Kinase and cancer metabolism”

- An increase in pyruvate kinase (PK) M2 (embryonic form) has been found in cancer cells.
- PKM2 inhibits the last step of glycolysis, thus having an anabolic effect on the cell (characteristic of cancer cells to undergo anabolism)
- Cancer cells have an increased glucose uptake that is also responsible for the anabolic effects observed.
- Mutations in PI 3K are responsible for the increased transport of glucose across the plasma membrane.
- Phosphoglycerinedehydrogenase (PHGDH) is a product of the PHGDH gene that is now known to oncogenic activity.
- Focal amplifications of this gene is increased in cancer where it then causes the diversion of glucose into serine synthesis.
- If PHGDH is overexpressed in breast cancer epithelial cells, it results in their transformation to malignancy.

**September 20, 2010**

Talks of interest and of relevance to my research work:

**Almut Shulze (London Research Institute, UK)**

“Regulation of cell metabolism by PI3-Kinase/Akt signalling and its role in cell growth and transformation”

- Again, the anabolic process emerges as an important characteristic of the cancer cell.
- The PI3-K pathways is frequently activated in cancer due to mutations/disruptions in molecules responsible for its control.
- Steroid response element binding protein (SREBP) is a transcription factor responsible for the activation of genes involved in fatty acid and cholesterol biosynthesis.
° Akt, a molecule that is downstream and under the control of PI3-K was observed to cause the activation of SREBP.
° Significance: PI-3K pathway is upregulated in many cancers, thus causing increased expression of Akt and thus SREBP.
° Fatty acid and cholesterol biosynthesis increases leading to anabolism of the cell.
° Confirmed by knocking-out SREBP:
° Cell growth is prevented where a decrease cell size, but not cell number, is observed.

September 20, 2010

Reuben J. Shaw (Salk Institute for Biological Studies, California)

“The AMPK pathway coordinates cell growth and metabolism”
° AMP-activated protein kinase K (AMPK) is an ‘energy sensing protein’ that plays an active role in controlling autophagy, glycolysis and glucose uptake and mitochondrial respiration.
° AMPK is a substrate for the tumour suppressor protein LKB-1.
° K/O of LKB-1 induces tumours and metabolic disease within a week in vivo.
° Autophagy: Loss of ULK-1 (ATG1) inhibits autophagy.
° AMPK was observed to control activity of ULK-1 due to the discovery of 4 binding sites on the ULK-1 molecule.
° K/O of AMPK causes the expression of p62 (cargo protein) to increase dramatically.
° Mitophagy: p62 is required for mitophagy and serves as a marker for degradation (-/- ULK-1 leads to altered/abnormal mitochondria)
° NB! siRNA ATG5 in serum starvation sensitises cancer cells to undergo apoptosis at an earlier stage.

September 20, 2010

Chris Bunce (University of Birmingham, UK)

“Proton NMR-based metabolic signatures of response to treatment in urine and serum of myeloma patients”
° Myeloma: Cancer of the bone marrow plasma cells.
° These plasma cells still secrete immunoglobins.
Metabolomics used as a method to determine changes in composition of metabolites in urine and serum samples of patients before and after treatment.

NB! ‘perfect samples’ are not realistic in a routine clinical practice.

**September 20, 2010**

Eileen White (The Cancer Institute of New Jersey, US)

“Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis”

- Autophagy provides flexibility during starvation through the maintenance of bioenergetics.
- Has a ‘garbage disposal function’ because the accumulation of unfolded proteins/organelles is toxic to the cell.
- Deficiency in autophagy → dramatic phenotype with damaged organelles and abnormal mitochondria is observed as well as DNA damage and death through apoptosis.
- *In vivo*, the activation of autophagy is seen in the hypoxic regions of the tumour.
- NB! Oncogene activation and thus malignancy leads to an ‘autophagic addiction’ in the cell.
- Ras activation increases autophagy above basal levels, but at the same time activates mTOR: The ability of autophagy levels to remain increased despite mTOR activity is due to the fact that autophagy induction by Ras completely overrides the inhibitory effect of mTOR.
- NB! Methods included a lot of immunology for cleaved caspase-3, p62 and ubiquitin.
- Focus changes to p62:
  - P62 binds to a polyubiquinated protein and to LC3 allowing LC3 to be shuttled to the autophagosome.
  - -/- p62 impairs tumorigenesis
  - During starvation, p62 and ATG5 are required for survival.
  - My study: look for abnormal mitochondria using TEM in siRNA ATG5 cells?
- Is autophagy required for mitophagy to take place? YES (Used PARKIN to track mitochondria).
- Found that mitochondria are required to survive starvation, and that uncoupling mitochondria prevents starvation.
- Autophagic defects cause a decrease in citrate metabolites which are required for lipid metabolism and cause an impairment in mitochondrial respiration and thus oxygen consumption.
**September 20, 2010**

**Kelvin Cain (University of Leicester, UK)**

“Metabolic reprogramming modulates tumour cell sensitivity in both intrinsic and death receptor- induced apoptotic cell death”

- 2-deoxyglucose (2-DG) potentiates trail-induced caspase activation (extrinsic apoptotic cell death) (Ben might find interesting)
- NB!! Levels of membrane bound vs. cytoplasmic Bax and cytochrome-c are VASTLY different when protein levels are examined through Western blot techniques (keep in mind)
- Pro-caspase-9, when detected with a Western blot is observed as 3 bands, indicating the presence of apoptosomes (interesting!)

**September 20, 2010**

**Stefano Indraccolo (University of Padova, Italy)**

“The glycolytic phenotype of cancer cells: implications for response to anti-VEGF therapy”

- Highly glycolytic cancer cells had markedly reduced viability under severe hypoxic conditions compared to cells with low glycolytic activity.
- Anti- VEGF treatment caused a decrease in glucose levels and ATP exhaustion as revealed by metabolomic studies, as well as large necrotic areas and tumour regression.
- However, cancer cells less reliant on glycolysis as a source of ATP exhibited less necrosis and tumours did not regress.
- NB! Find out metabolic differences between HeLa and Caski cells.

**September 21, 2010**

**Chi V. Dang (John Hopkins University School of Medicine, US)**

“Myc, MicroRNAs, and targeting cancer metabolism”

- Myc is responsible for ribosomal biogenesis, inducing OXPHOS and glutamine metabolism, as well as increases cellular mass (anabolism).
Not all tumours follow the Warburg effect! Some cancers rely on glutamine metabolism and OXPHOS.

Important finding: Myc controls glutamine catabolism and myc-driven tumours rely on glucose AND glutamine.

September 21, 2010

Gregg L. Semenza (John Hopkins University School of Medicine, US)

“HIF-1: Upstream and downstream of tumour metabolism”

- Hypoxia is found in advanced human cancer.
- Cells that are hypoxic are observed to be resistant to chemotherapy.
- A lack of oxygen seems to enable these cells to become metastatic and resulting patient mortality is high.

HIF-1

\[ \text{EPO} \rightarrow \text{VEGF} \rightarrow \text{IGF-2} \rightarrow \text{Glut} \]

\[ \uparrow \text{O}_2 \text{ delivery} \]

- HIF-1 increases in response to:
  - Viral oncoprotein expression \text{HPV E6/E7}
  - ROS
  - PI3-K/Akt and mTor activation
- NB!! HIF-1 was observed to be increased in ALL STAGES of cervical cancer

September 21, 2010

Jaques Pouyssegur (Center National de la Recherche Scientifique, France)

“Tumour hypoxia and metabolic acidosis”

- The beclin-1-Bcl2 complex is one of low affinity and regulates beclin-1-dependant autophagy.
- Bnip3 competes with beclin-1 for Bcl-2 thereby causing the dissociation of beclin-1, where it can begin its function in inducing autophagy.
° Bcl-2-Bnip3 complex (low affinity) inhibits cell death through apoptosis.
° Hypoxia induces HIF-1-dependant autophagy and mitophagy via Bnips.