Epigenetic Modulation of Ovarian Cancer Cells

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Objective

- Recap what do we know already
- Understand Epigenetics in cancer
- Analyse the design, methods and results
- Discuss future works

What do we know so far

- A common feature of Cancer is:
  - high acid load in the tumour microenvironment
- To counteract cancer cell uses no. of mechanisms
  - Family of two pore domain potassium (K+)channels
- Previous studies have proven increased expression of voltage gated and K_2P in Ovarian cancer.

Epigenetics and Cancer

- Epigenetics is the study of mechanism that alter gene expression without changing the primary DNA nucleotide sequence.

- Several different types
  - DNA methylation,
  - histone modifications

DNA Methylation and Cancer

- Normally,
  - 80% of all nucleotides are found to be methylated in mammalian genomes.
  - Rest 20%, CpG islands remain hypomethylated.
- In Cancer,
  - the CpG islands are more methylated
  - Rest of the genome, are hypomethylated.

Hypothesis

Epigenetic modulation in ovarian cancer cells maintained in altered microenvironment affects cancer cell proliferation through pH-sensitive membrane proteins that include K^+ channels.

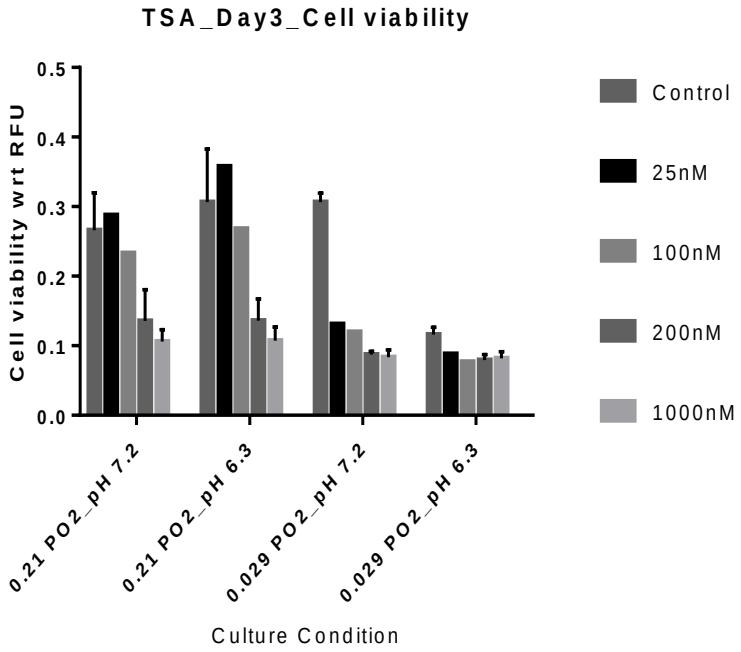
Aim

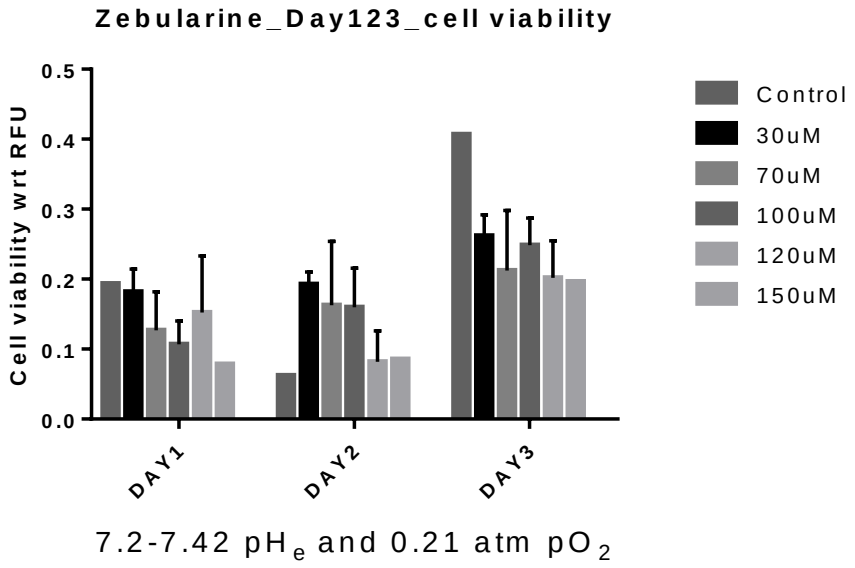
- Establish the relation between low pH_e and high pH_i and pharmacologic epigenetic modulation of SK-OV-3 cell lines.
- To investigate the level of expression of K^+ channels in cancer cells after epigenetic modulation
- Selection and designing of gene specific primers compatible for bisulphite converted genomic DNA.

Design: Methods and Materials

- Proliferation of SKOV3 cells at pH_e 6.3 and pH_e 7.5 is assessed after exposing them to epigenetic modulation
  - 5-Azacytidine
  - Zebularine
  - Trichostatin -A
- Mechanism of action

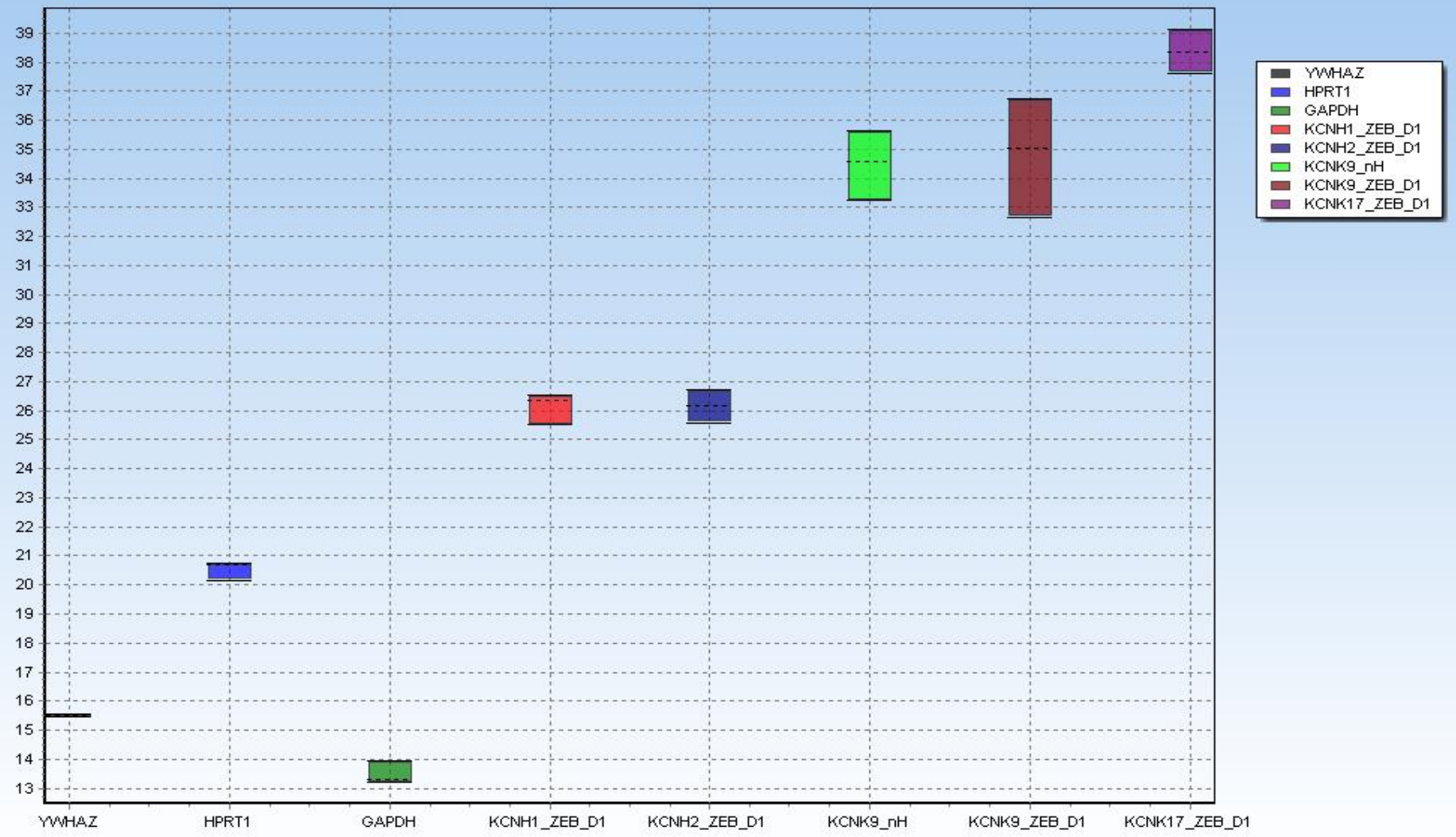
Results





7.2-7.42 pH_e and 0.21 atm pO_2

Relative Gene expression of K^+Channel in presence and absence of ZEB

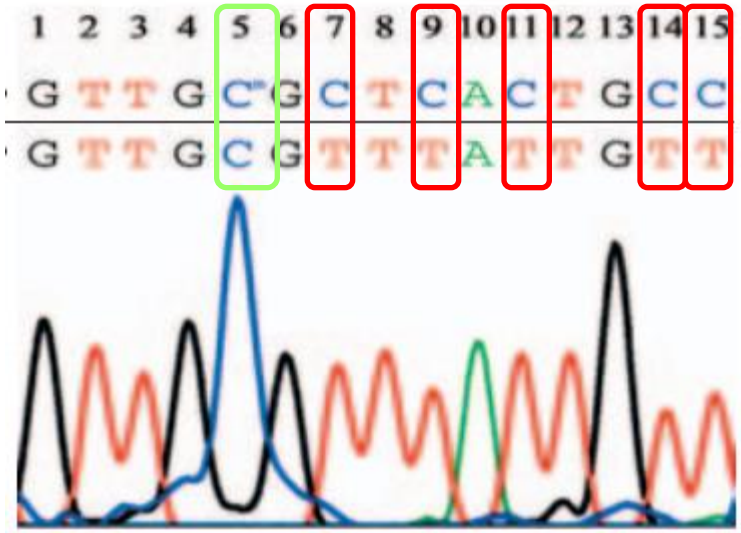


Further Works

- Detection of CpG islands adjacent to promoter region of *KCNK9, KCNK17, KCNK10* genes coding for TASK3, TALK2 *and* TREK2.
- Designing two sets of primers (compatible to Bisulphite conversion) for nested PCR reactions.

Bisulphite Treatment and conversion

- DNA with epigenetically modulated (C^mpG) was processed.
- The methylated cytosine remains intact.
- Unmethylated cytosines were completely converted into uracil following bisulfite treatment
- Detected as thymine following PCR.



Any questions?

Summary

- K2P channels are overexpressed in Ovarian cancer
- Epigenetic modulation alters their expression
- Does the modulation alter the methylation status of the promoter region?

Acknowledgement

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