Prostate cancer is one of the most common cancers in the world, with approximately 12.9% of men being diagnosed with the disease at some point in their lives. One gene associated with prostate cancer is *RASSF1A* (Ras Associated Domain Family 1A), which is a tumour suppressor gene [1]. RASSF1A acts in many cell growth control pathways and can help induce apoptosis or cell cycle arrest [2]. Patients with prostate cancer exhibit hypermethylated RASSF1A promoter regions, which has been seen to be associated with silencing of the gene [1,3,4]. In general, genome wide demethylation is seen with age, however age association hypermethylation has been shown to overlap with hypermethylation of promoters in cancer associated genes [5]. It is well known that the risk of developing prostate cancer increases with age. However, it is unknown *if the RASSF1A gene promoter is targeted for hypermethylation as age increases.*

My **primary goal** is to identify if/why the RASSF1A gene promoter becomes targeted for hypermethylation with age.

My **hypothesis** is that the RASSF1A gene promoter is targeted for hypermethylation with age.

My **long term goal** is to better understand the role of hypermethylation of the RASSF1A gene promoter and how it affects the genes regulation in prostate and other cancers.

**Aim 1:** Establish the level of conservation in the RASSF1A gene promoter between humans and other mammal homologs and then carry out bisulfite sequencing to establish methylation levels in the RASSF1A promoter homologs.

**Rationale:** To identify if homologs (mice, rats and chimpanzee’s) share conserved regions when aligned and to establish if they also show the same pattern of hypermethylation.

**Approach:** I will use Clustal Omega to align the FASTA sequences. For the bisulfite sequencing, I would treat the DNA with bisulfite and then sequence. The level of methylation would be based on the number of remaining cytosine residues as unmethylated cytosine’s would be converted to uracil.

**Hypothesis:** I hypothesise that the homologs will have very similar conserved regions but the methylation patterns will differ between species.

**Aim 2:** Use CRISPR/Cas9 to target RASSF1A promoter region for demethylation in mice with prostate cancer and compare RASSF1A expression in treated mice to wildtype mice.

**Rationale:** To demethylate the promoter region of RASSF1A, to see if the gene is un-silenced and the effect an active RASSF1A has on prostate cancer.

**Approach:** I would fuse Tet1-CD with Cas9 and create sgRNA to guide the system to the promoter region of RASSF1A. The system would be delivered by injection into the prostates of adult male mice with prostate cancer. I will use a western blot to measure protein expression levels, before and after treatment.

**Hypothesis:** Before treatment the mice with prostate cancer will show lower levels of RASSF1A expression than the wildtype mice. After treatment they will show the same level of RASSF1A expression.

[1] Ge, YZ., Xu, LW., Jia, RP. et al. 2014. The association between RASSF1A promoter methylation and prostate cancer: evidence from 19 published studies. *Tumour Biol*. **35**: 3881
[2] Donninger, H. Vos, M.D. & Clark, G.J. 2007. The RASSF1A tumour suppressor. *Journal of Cell Science*. **120**: 3163-3172.
[3] Yegnasubramanian, S., Kowalski, J., Gonzalgo, M.L. et al. 2004. Hypermethylation of GpG islands in primary and metastatic human prostate cancer. *Cancer Research*. **64**(6): 1975-1986.

[4] Baylin, S.B. & Herman, J.G. 2000. DNA hypermethylation and tumorigenesis. Epigenetics joins genetics. *Trends in Genetics.* **16**(4): 168-174

# [5] Teschendorff, E.A., West, J. & Beck, S. 2013. Age-associated epigenetic drift: implications, and a case of epigenetic thrift? *Human Molecular Genetics.* 22: 7-15