

Investigating the Role of TET Proteins in Pancreatic Cancer Stem Cells

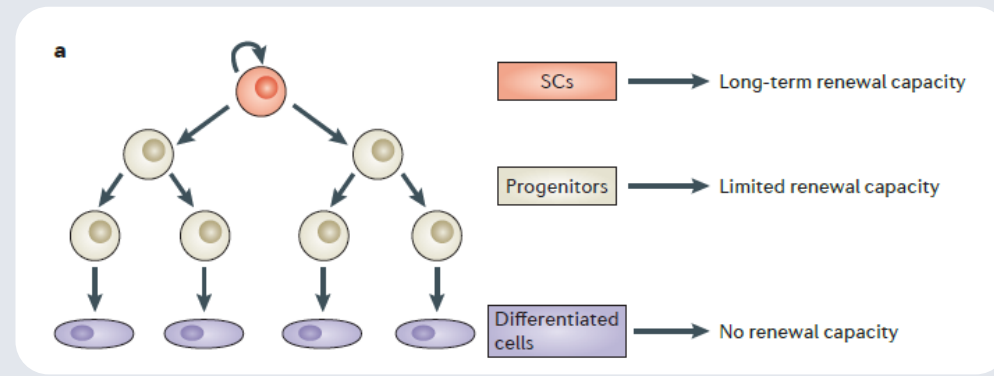
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Cancer Stem Cells

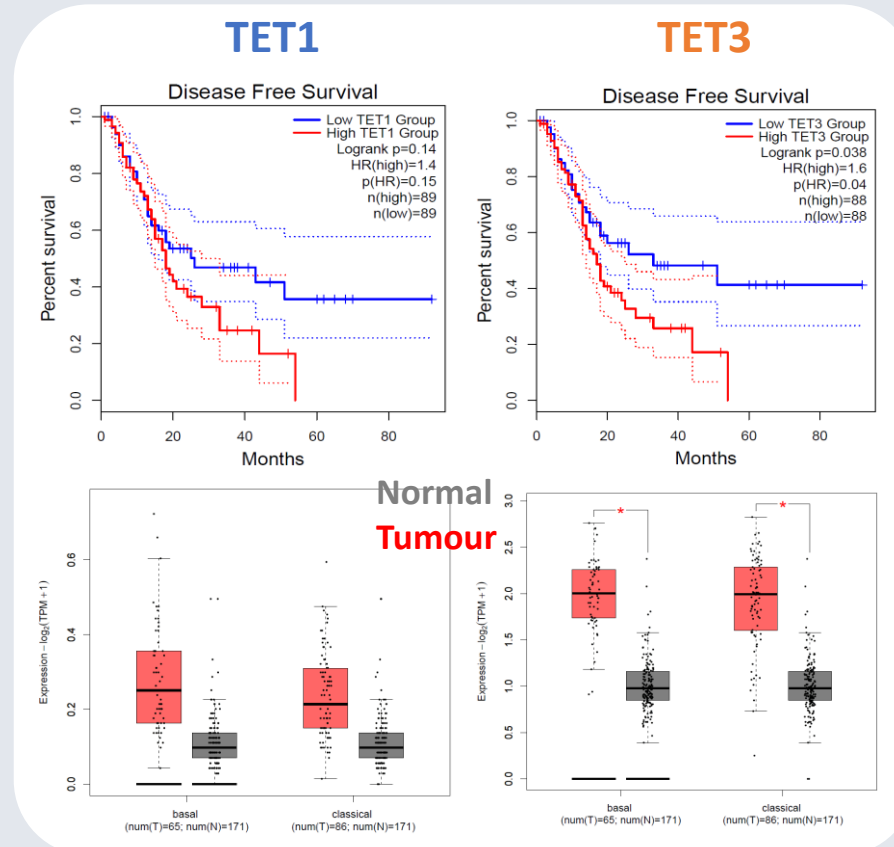
- Minority sub-population approx., 0.01-5%
- Tumour-initiating
- Self-renewing
- Anoikis-resistant
- Drug-resistant



Beck B, Blanpain C. Unravelling cancer stem cell potential. *Nat Rev Cancer*. 2013 Oct;13(10):727-38. doi: 10.1038/nrc3597. PMID: 24060864.

Minority tumour-initiating population with characteristics of stem cells identified in PDAC (Li 2007, Herman 2007, Lonardo 2011)

- CSC phenotype is plastic
- TET1 important in induced pluripotency
- Ten-Eleven TF (TET) enzymes oxidise 5mC bases to 5-hmC (5-hydroxymethylcytosine) to reverse gene silencing
- Further oxidised to 5-fC (5-formylcytosine) and 5-caC (5-carboxylcytosine) to remove modification

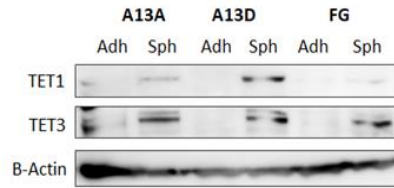
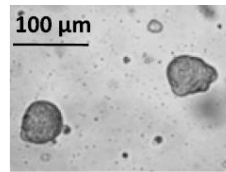
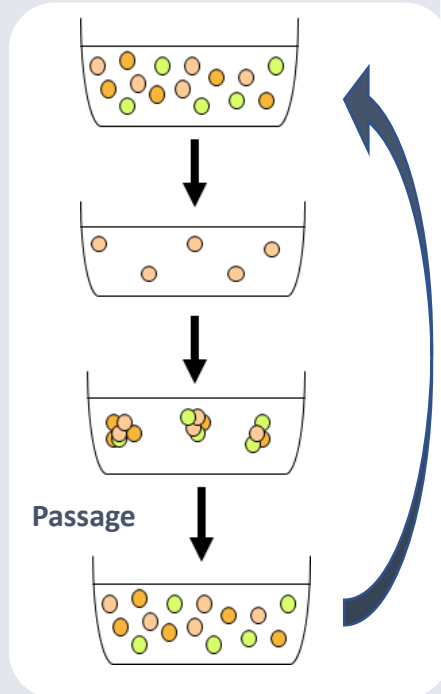


Key CSC Genes are Hydroxymethylated in Tumourspheres

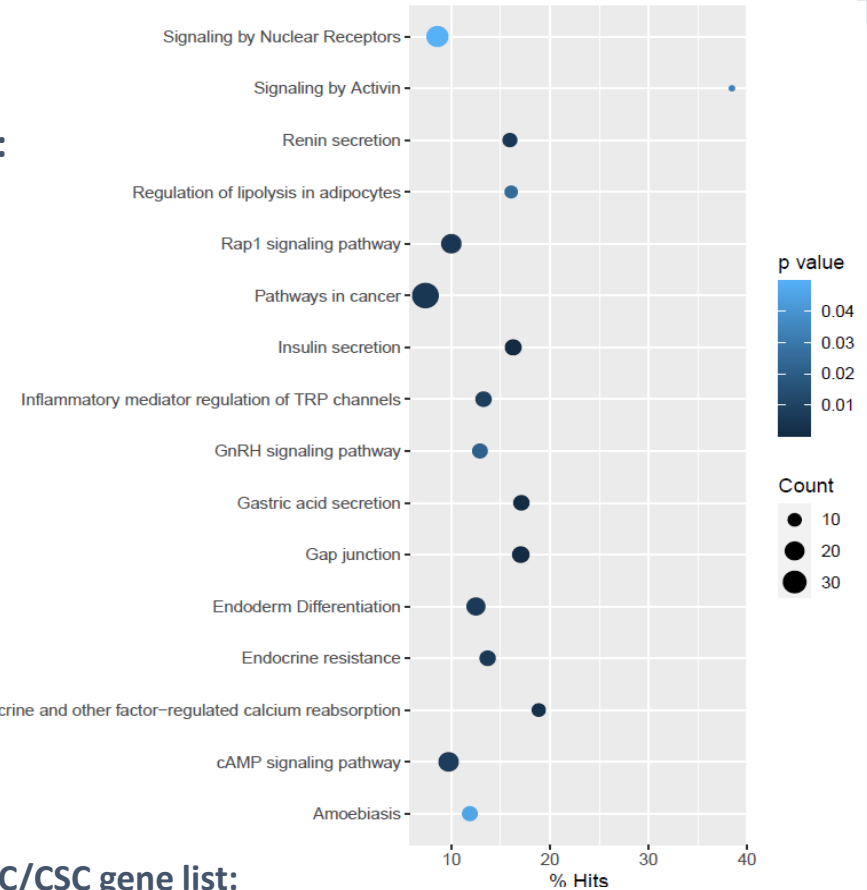
Tumoursphere Culture

Cells seeded at low density to select for:

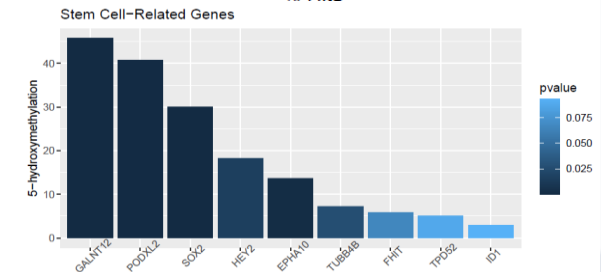
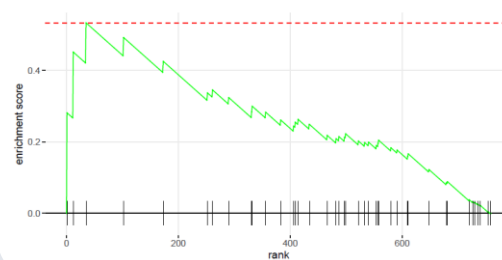
1. Anoikis resistance,
2. Proliferation from single cells
3. Self-renewal (Passage)



Oxidative Bisulphite Sequencing:

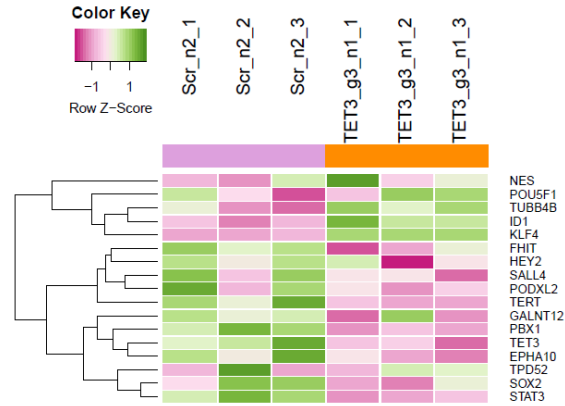
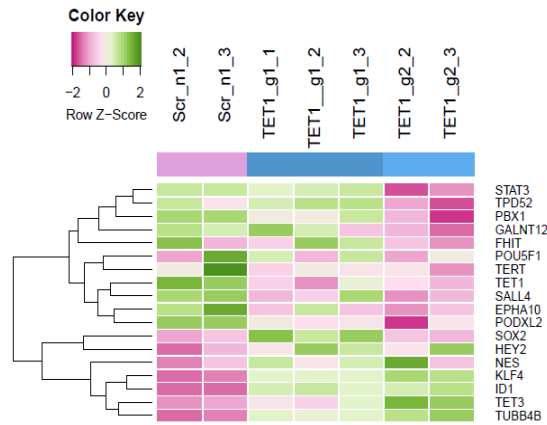


GSEA using ESC/CSC gene list:

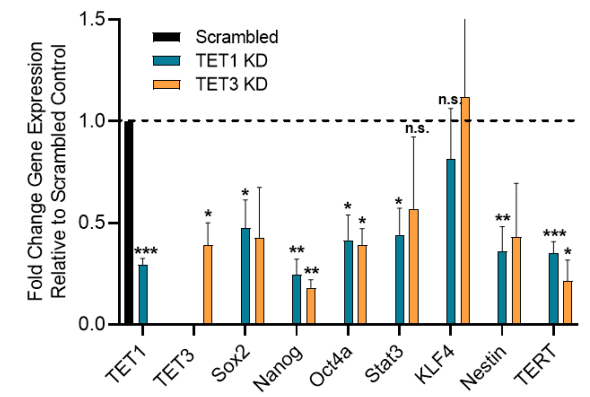


CRISPRi TET1 or TET3 Knockdown Impairs CSC Function

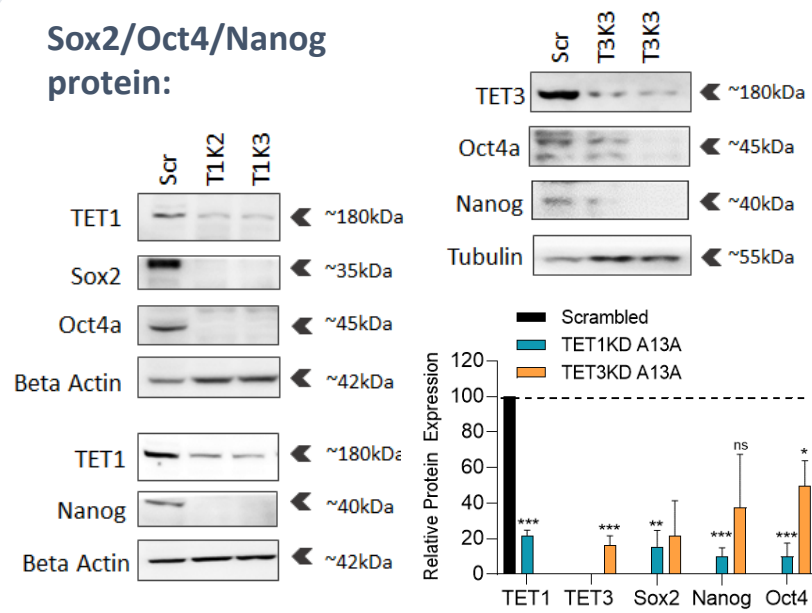
Pluripotency-Associated genes expression, RNASeq:



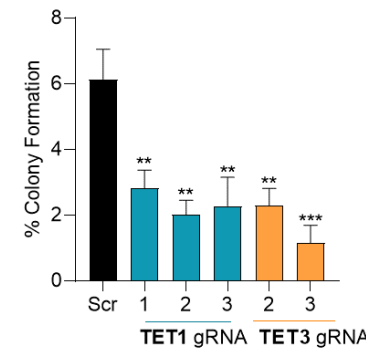
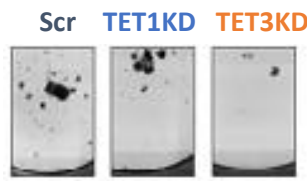
Pluripotency-Associated gene expression, qPCR:



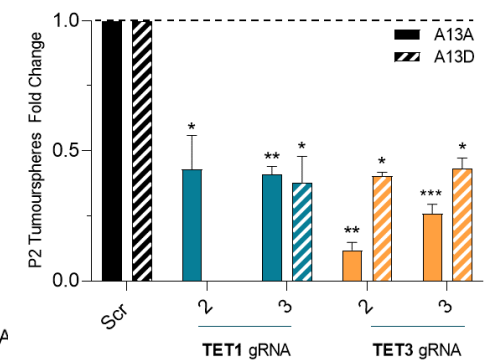
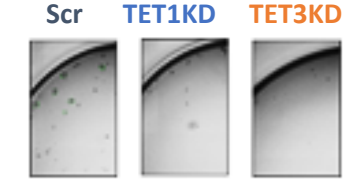
Sox2/Oct4/Nanog protein:



Colony Formation

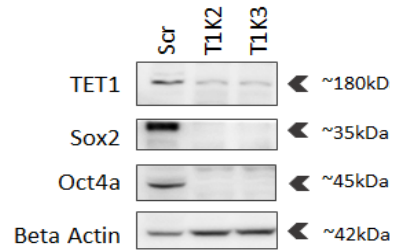


Tumoursphere Formation

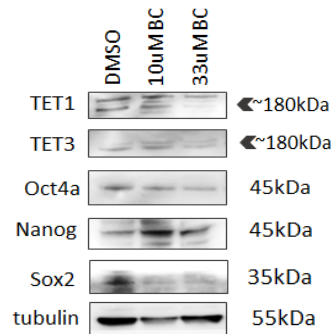


TET1 and TET3 Mechanism of Action

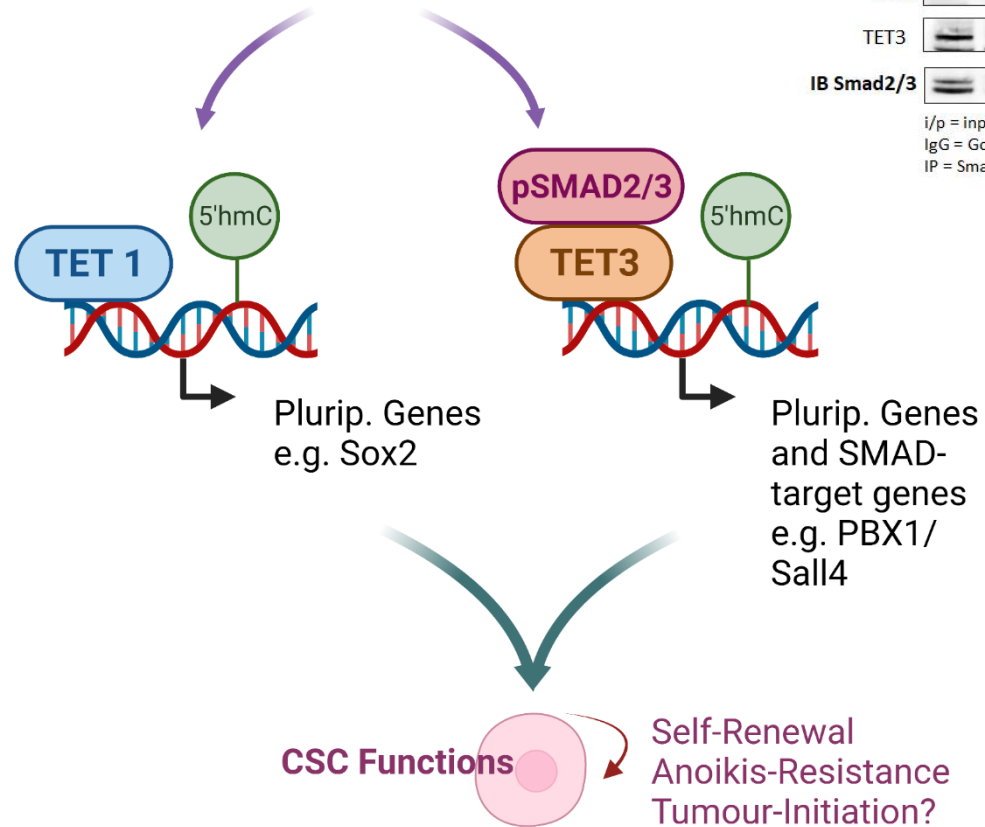
TET1 Knockdown:



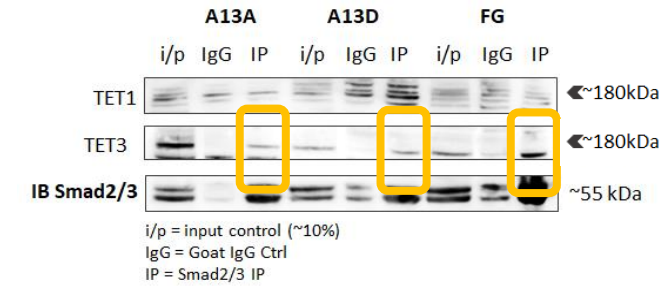
Bobcat339 (TET1/2 Inhibitor):



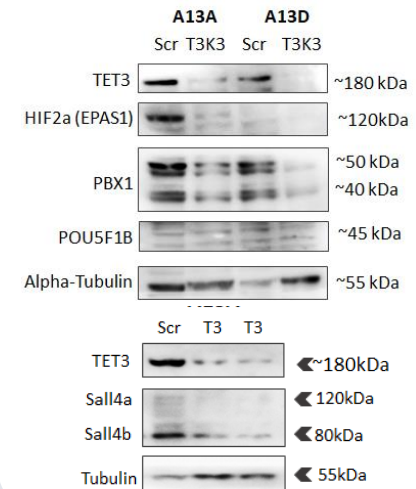
kRAS? Hyperglycaemia?



Nuclear Complex CoIP; Smad2/3 Pulldown:



TET3 ChIP Targets:



Acknowledgements

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