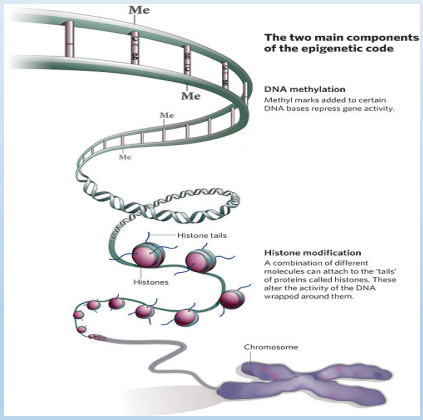
Marcadores epigenéticos: A nova era da genética molecular



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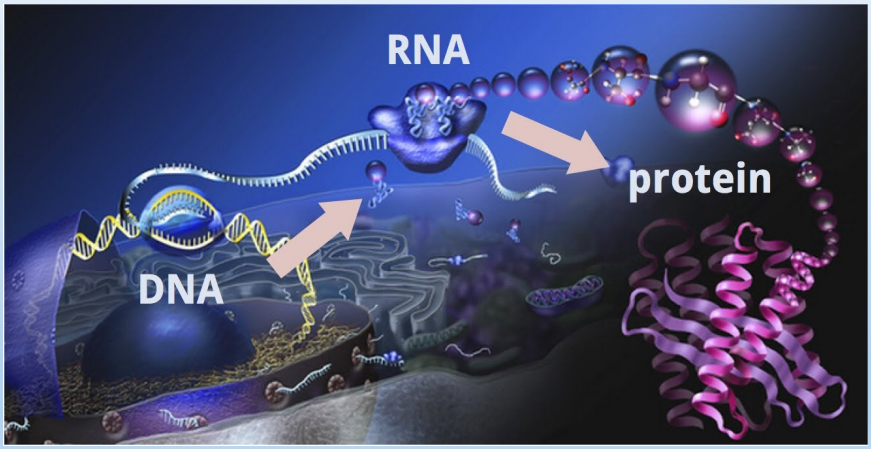
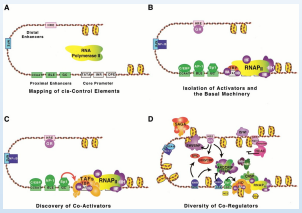
Centro Académico de Investigação e Formação Biomédica do Algarve

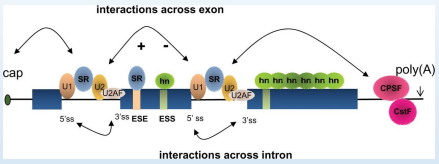
Pedro Castelo-Branco

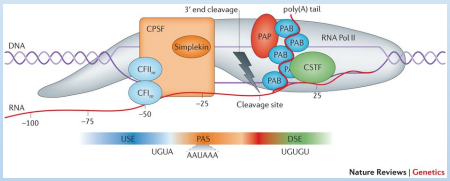
From DNA to Protein

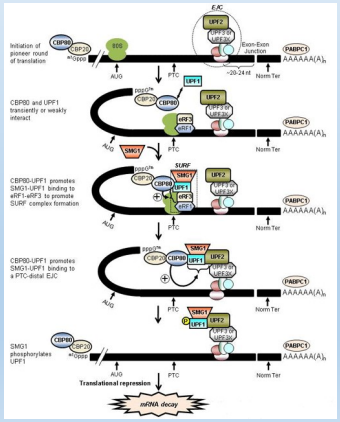


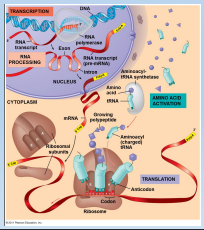
From DNA to Protein











Epigenetics

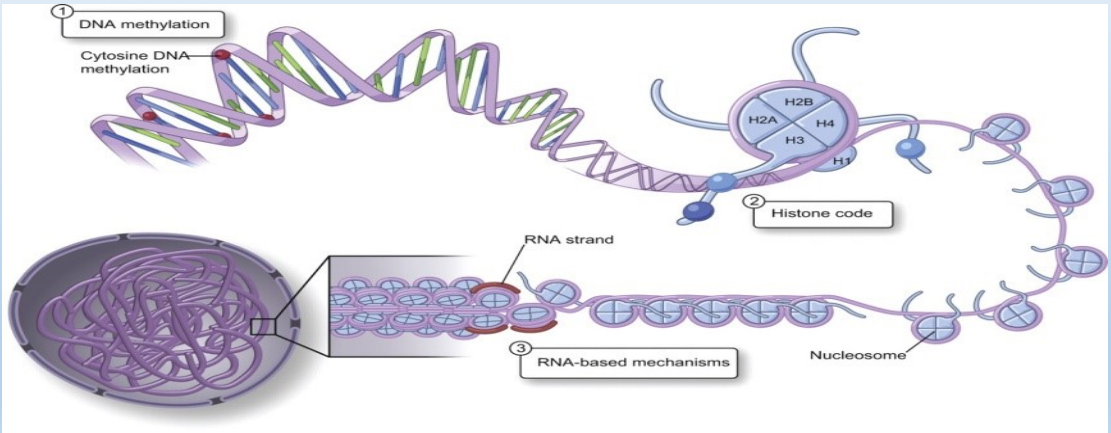
from mechanisms to disease

Environmentally-mediated and widespread changes that **affect gene expression without modifying the DNA sequence**

Epigenetic modifications includes:

- DNA methylation
- Histone modification
- Small non-coding RNAs

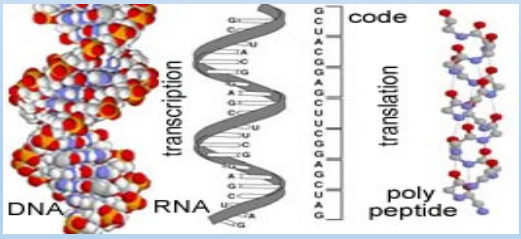
Epigenetic changes



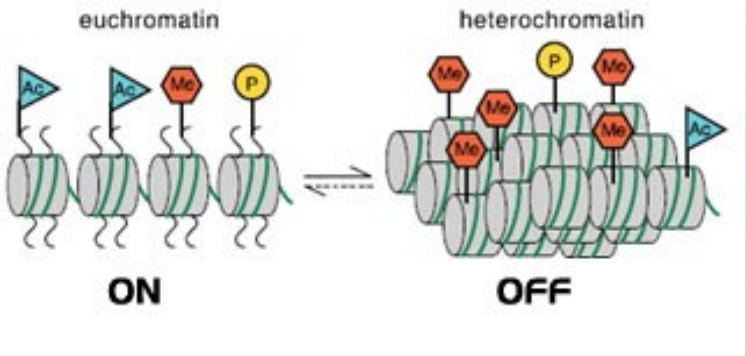
http://www.intechopen.com/source/html/44560/media/image1.jpeg

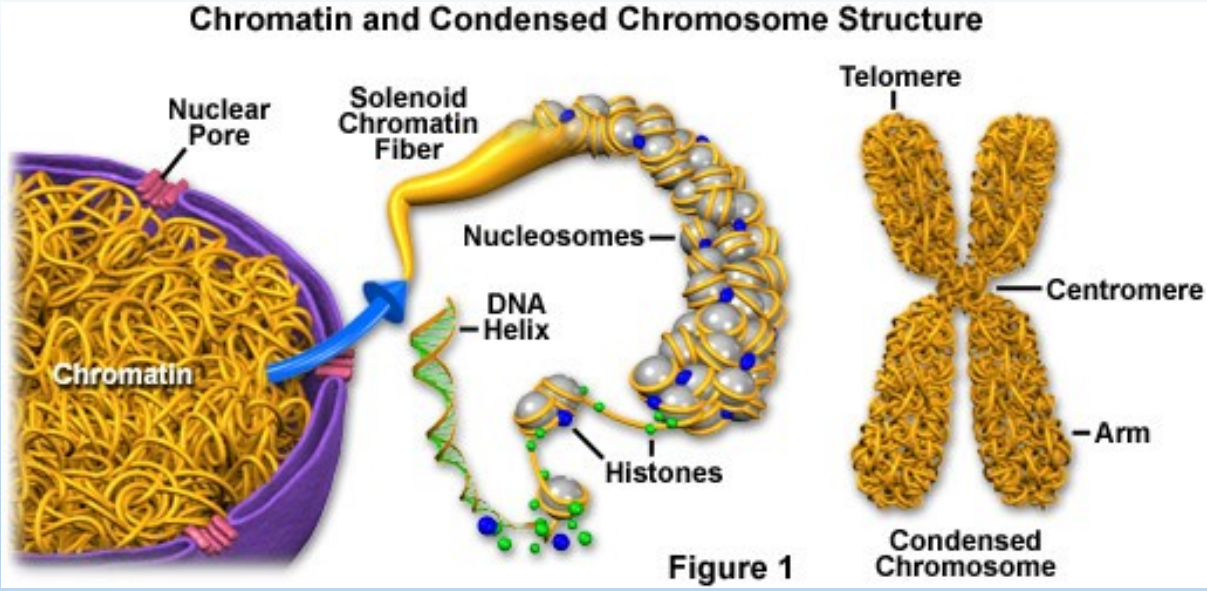
Chromatin Structure

- Every cell in our body contains exactly the **same genome**, however, inside the individual cells **some genes are activated while others are silenced.**

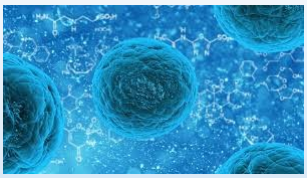


Chromatin Structure





micro.magnet.fsu.edu



2 meters DNA per cell

X

30 trillion cells

= **60 000 000 000 kilometers**

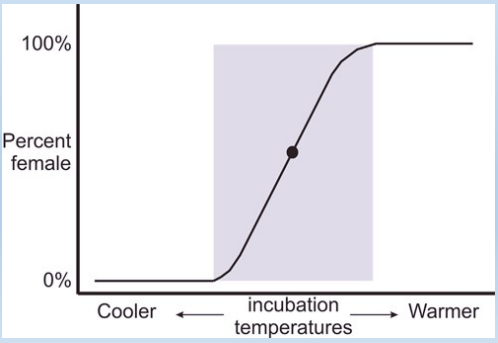
Distance Hearth to the Sun: 150 000 000 kilometers

Epigenetics: Sex determination

- **Turtles**

Sex phenotype determined by environmental conditions – **temperature**

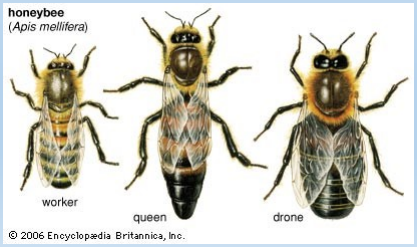
Differences in temperature alter the percentage of **DNA methylation** of the *aromatase (estrogen synthetase)* promoter



Diaz-Hernadez, et al. Developmental Biology, volume 408, Issue 1

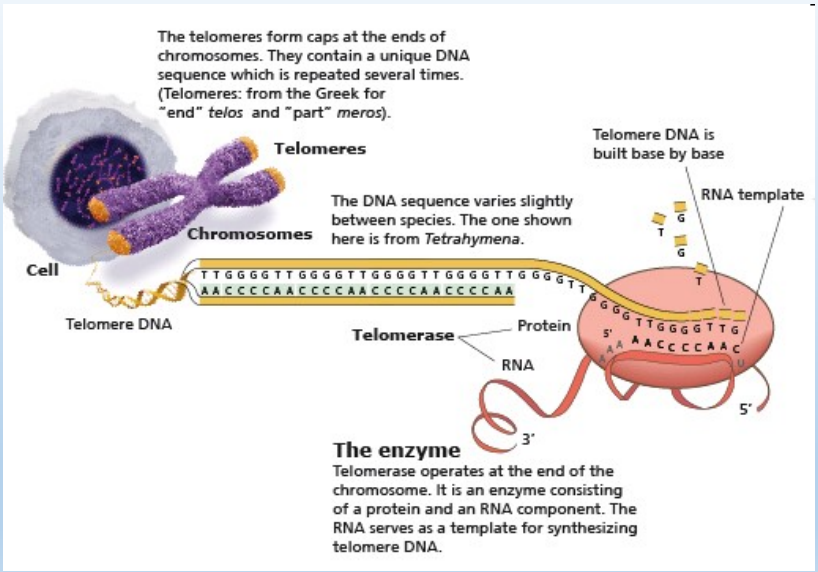
Epigenetics: Phenotype / Longevity

- When ***Dnmt3*** **is turned "on,"** the queen genes are epigenetically silenced, and the larvae develop into the default **"worker" variety**.
- But when royal jelly turns ***Dnmt3*** **"off,"** the queen genes jump into action, turning the larvae into **queens.**



Queen bees live
10x longer
than worker bees!!!!!

Telomeres and Telomerase



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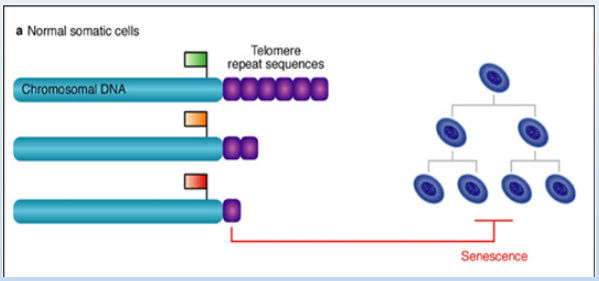
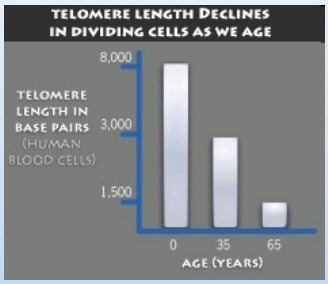
Aging





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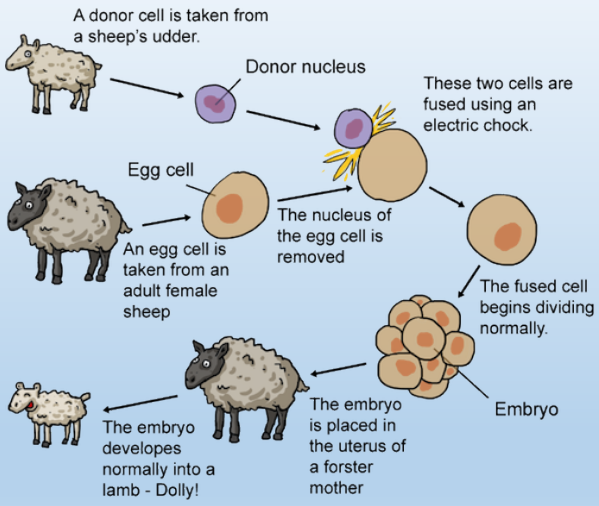
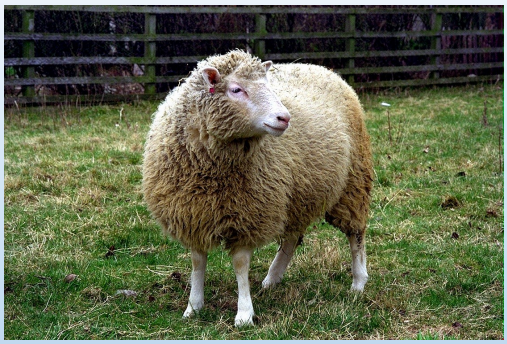
Aging – The Dolly story



Aging – The Dolly story

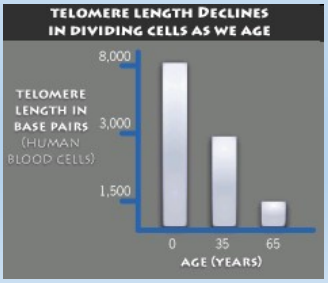


Dolly (5 July 1996 – 14 February 2003)

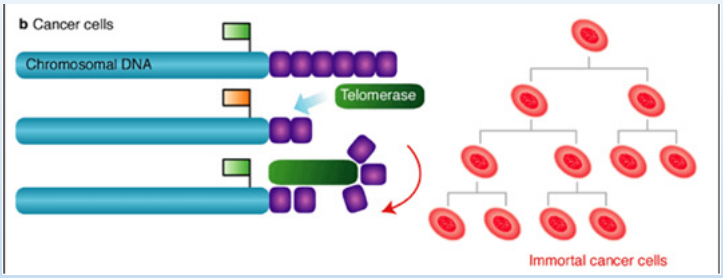
Fact: Sheep's life expectancy – 15 years.

Fact: Dolly's "Mother" Age – 6 years.

"Em 1999 foi divulgado na revista *Nature* que Dolly poderia tender a desenvolver formas de envelhecimento precoce, uma vez que os seus telómeros eram mais curtos que os das ovelhas normais."



Telomerase activation in cancer



Telomerase is active in 90% of cancers

Prognostic Factor in Cancer

Telomerase activity is also found in normal cells – embryonic stem cells, germ lines, lymphocytes.

Pergunta: Como é que as células cancerigenas activam a telomerase?

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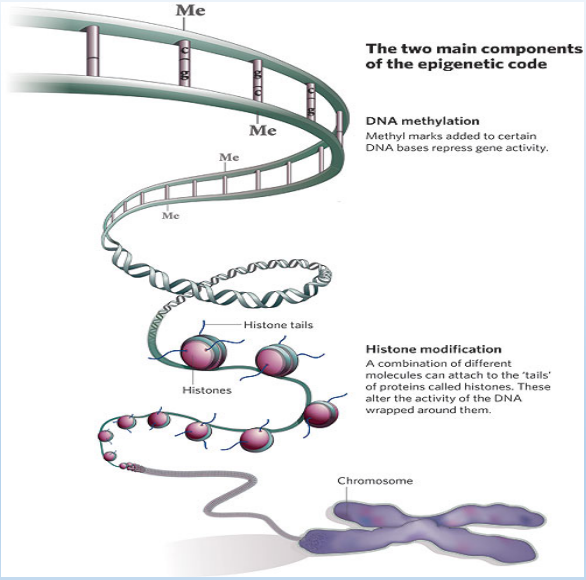
Mecanismo Epigenético



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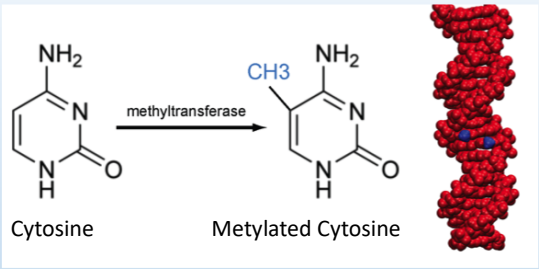
Qiu, Nature, 2006

DNA methylation



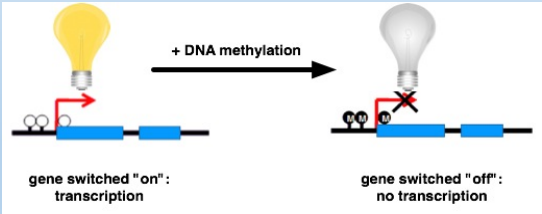
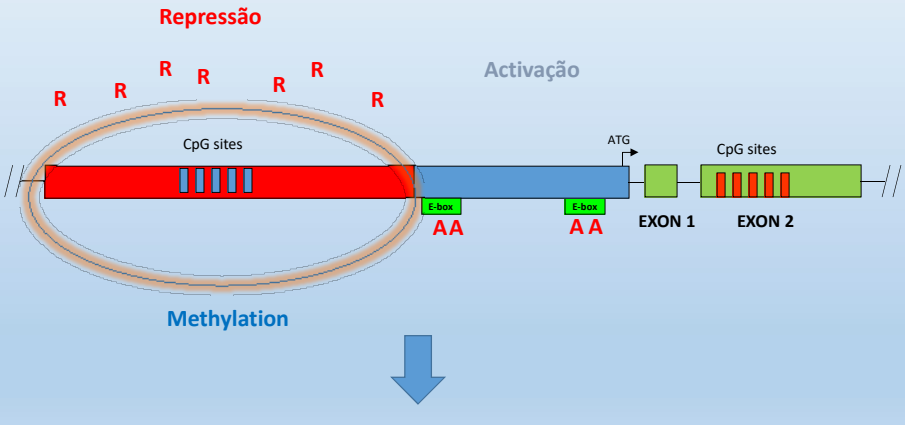


figure 1: Transcriptional silencing of gene promoters via DNA methylation

Metilação no promoter da TERT promove activação do gene



Telomerase activa no cancro !!!

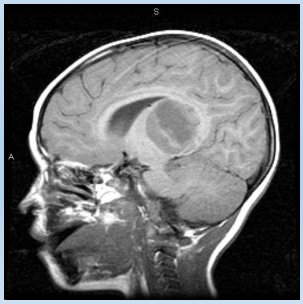
Implicações no diagnóstico e prognóstico do cancro

Articles

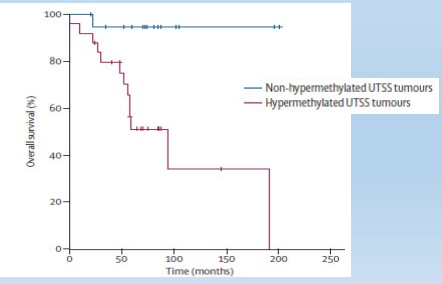
Lancet Oncol 2013; 14: 534–42

Methylation of the TERT promoter and risk stratification of childhood brain tumours: an integrative genomic and molecular study

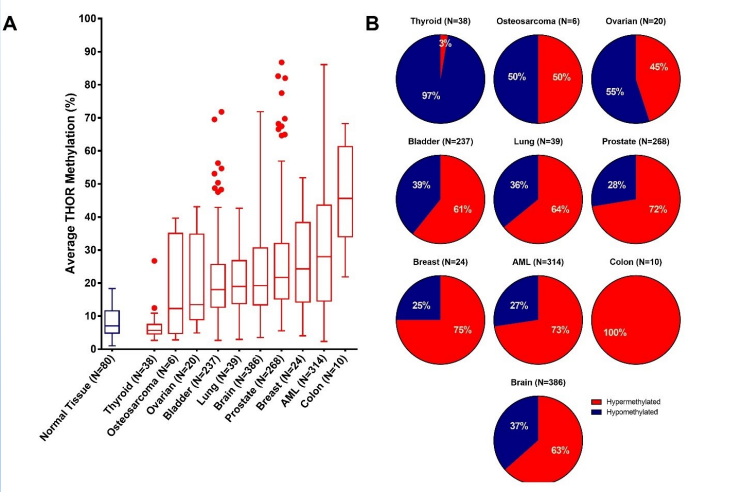
Pedro Castelo-Branco, Sanaa Choufani, Stephen Mack, Denis Gallagher, Cindy Zhang, Tatiana Lipman, Nataliya Zhukova, Erin J Walker, Dianna Martin, Diana Merino, Jonathan D Wasserman, Cynthia Elizabeth, Noa Alon, Libo Zhang, Volker Hovestadt, Marcel Kool, David T W Jones, Gelareh Zadeh, Sidney Croul, Cynthia Hawkins, Johann Hitzler, Jean C Y Wang, Sylvain Baruchel, Peter B Dirks, David Malkin, Stefan Pfister, Michael D Taylor, Rosanna Weksberg, Uri Tabori



Overal Survival – ependymoma patients

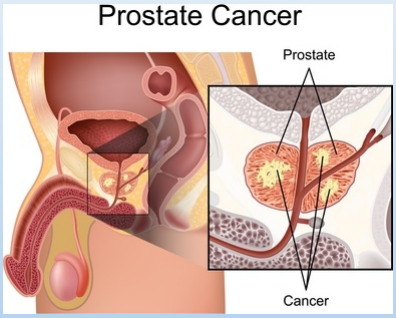


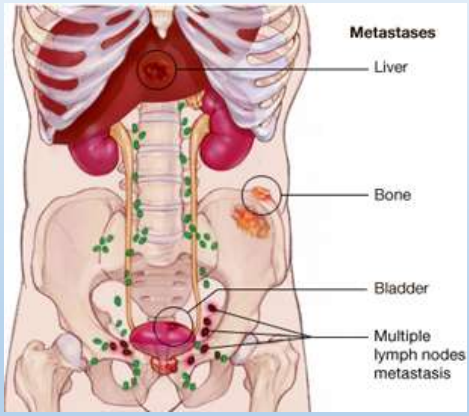
THOR is a pan-cancer biomarker



Dave Lee, et al. *Jour. Clin. Investigation 2018*

Prostate cancer – primary and metastatic





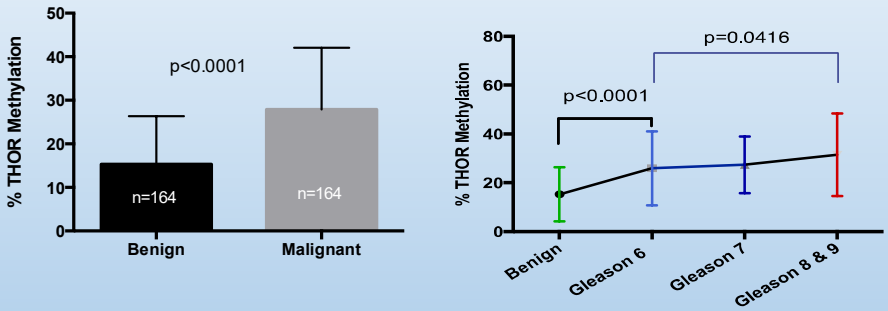
Prostate cancer – pathological grading

Gleason Sum of Primary and secondary histological patterns (such as Cell morphology and invasion) ex: Gleason 7 (4+3 or 3+4)

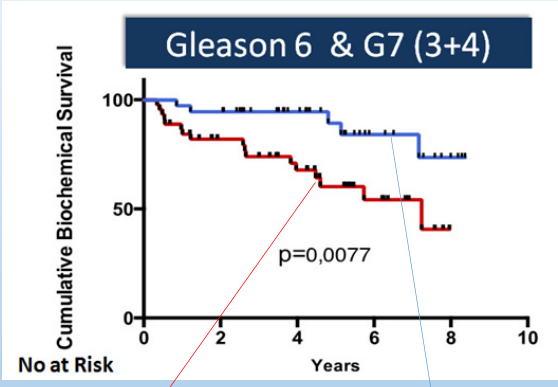
Gleason Score 2, 3, 4	Gleason Score 5, 6, 7	Gleason Score 8, 9, 10
Low-grade tumor	Medium-grade tumor	High-grade tumor
Slow Growth	Unpredictable Growth	Aggressive Growth
Least dangerous. Cells look most like normal prostate cells and are described as being "well-differentiated". Tends to be slow growing.	Intermediate cancers may behave like low-grade or high-grade cancers. The cells' behavior may depend on the volume of the cancer and the PSA level. This is the most common grade of prostate cancer.	High-grade cancers are usually very aggressive and quick to spread to the tissue surrounding the prostate. These cancer cells look least like normal prostate cells and are usually described as "poorly-differentiated".

Grey Area

THOR distinguishes benign and malignant prostate samples



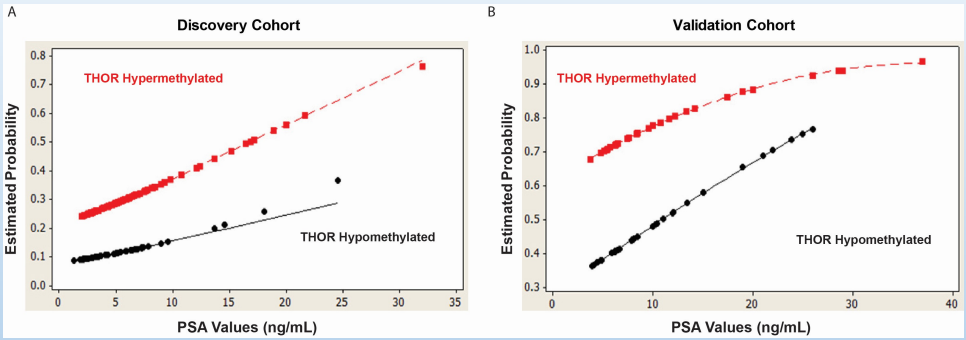
THOR defines two different populations of patients amongst lower Gleason scores



Treat immediately

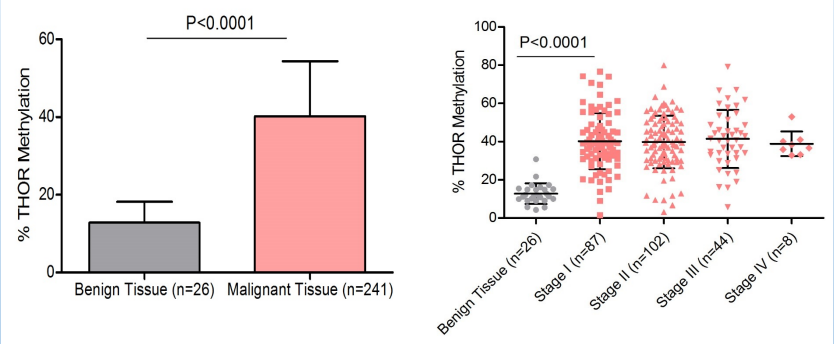
Hold Treatment

THOR significantly adds predictive value to Prostate Cancer recurrence.



Ricardo Leão et al *Oncotarget,* 2016

THOR is Diagnostic Biomarker in Breast Cancer



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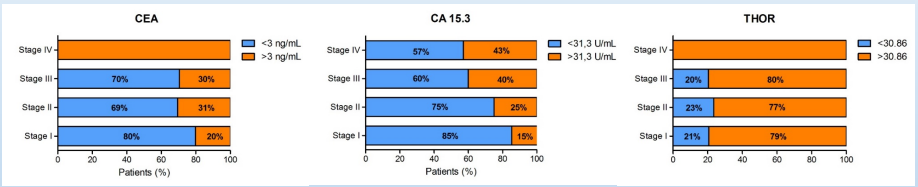
THOR compared to other biomarker for breast cancer diagnosis



✓ A high percentage of patients with invasive disease had normal values of CA 15.3 and CEA, but had pathological levels of THOR.

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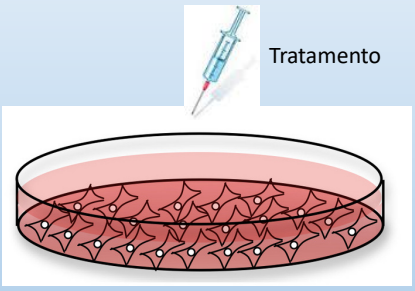
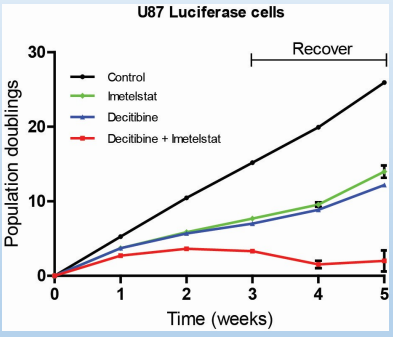
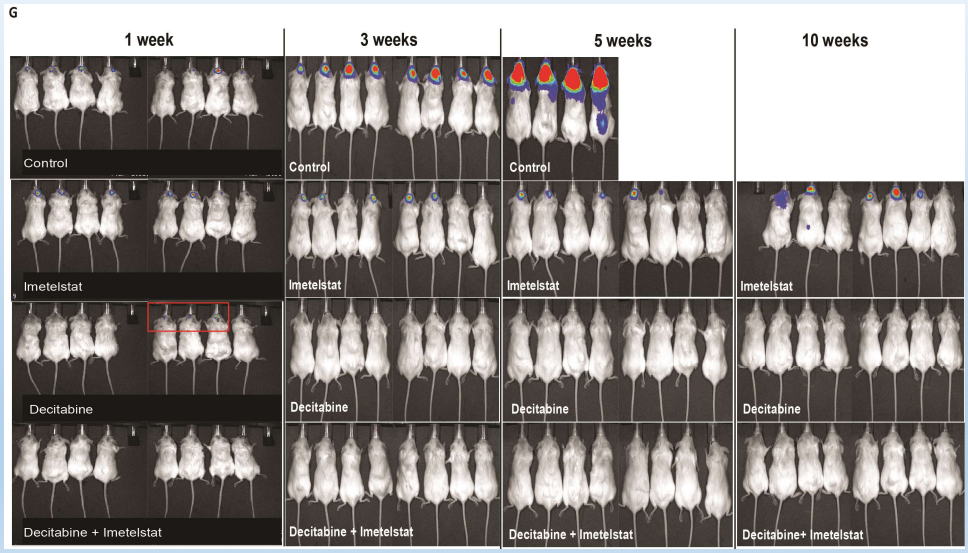
Implicações terapeuticas

Tratamento com agentes demetilantes inibe a divisão celular (in vitro)





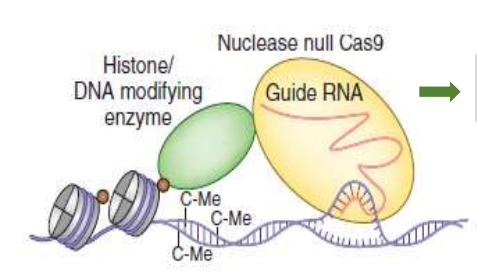
Tratamento com agentes demetilantes previne a recorrência tumoral (in vivo)



Targeted THOR demethylation using CRISPR/dCas9 system

CRISPR/dCas9 system

- Cas9 catalytic domain inactivated (dCas9) and function conferred by fusion to a novel effector domain, the catalytic component of TET1 demethylase enzyme.



Rusk N., *Nature Methods*, 2014

dCas9 fused with TET1 (dCas9-TET1) + **guide RNA**

↓

THOR demethylation

↓

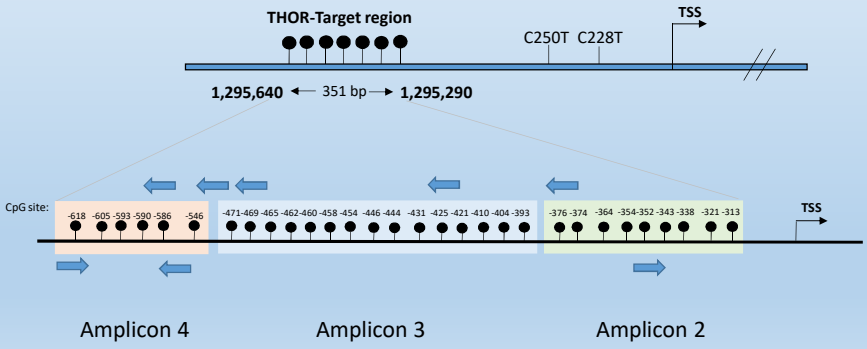
h*TERT* suppression

Joana Apolónio et al., *in prep.*

Scheme of h*TERT* promoter region for targeted demethylation

Benchling

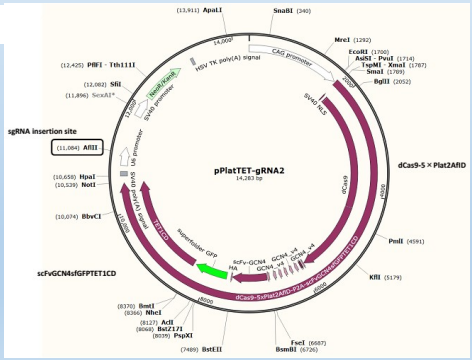
Human *TERT* locus
chr5, hg19



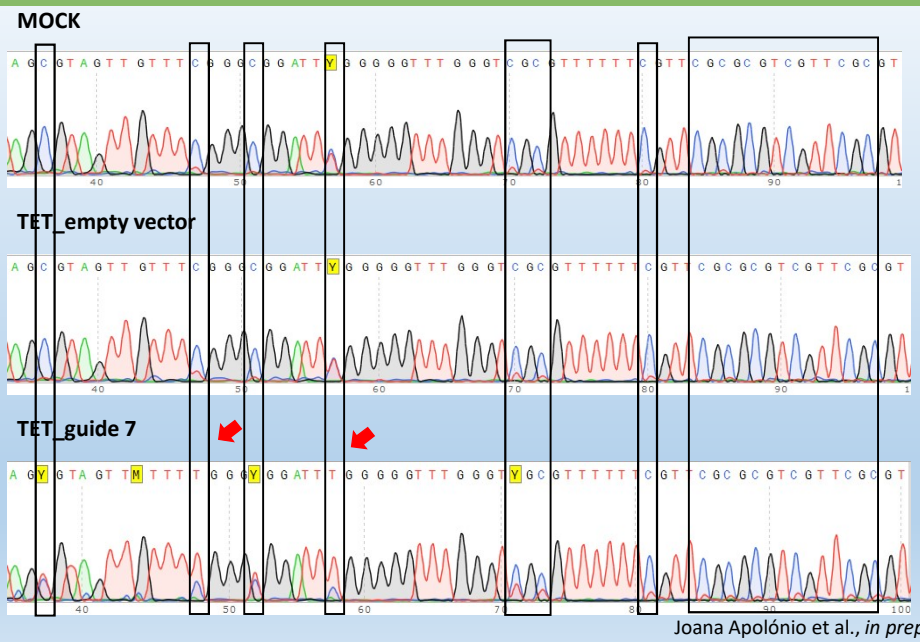
Amplicon 4 Amplicon 3 Amplicon 2

Joana Apolónio et al., *in prep.*

TET_guide plasmid



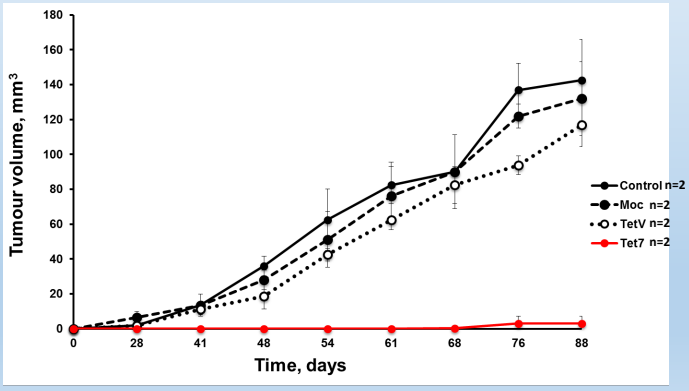
THOR demethyaltion



Joana Apolónio et al., *in prep.*

In vivo Pilot study
MCF-7 cells transfected with TET-g7

- Injection day: day 0
- Number of cells: 162K cells were cell sorted 48h post-transf and injected into the right leg of NOD/SCID mice
- Tumor size was monitored along time.



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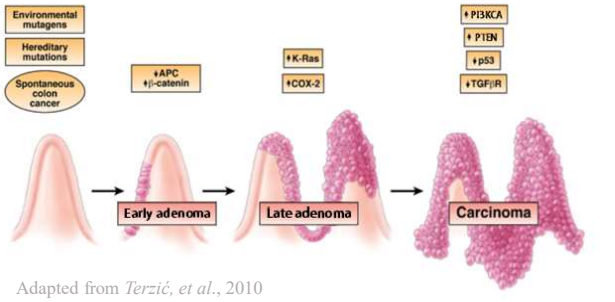
Colorectal cancer (CRC)

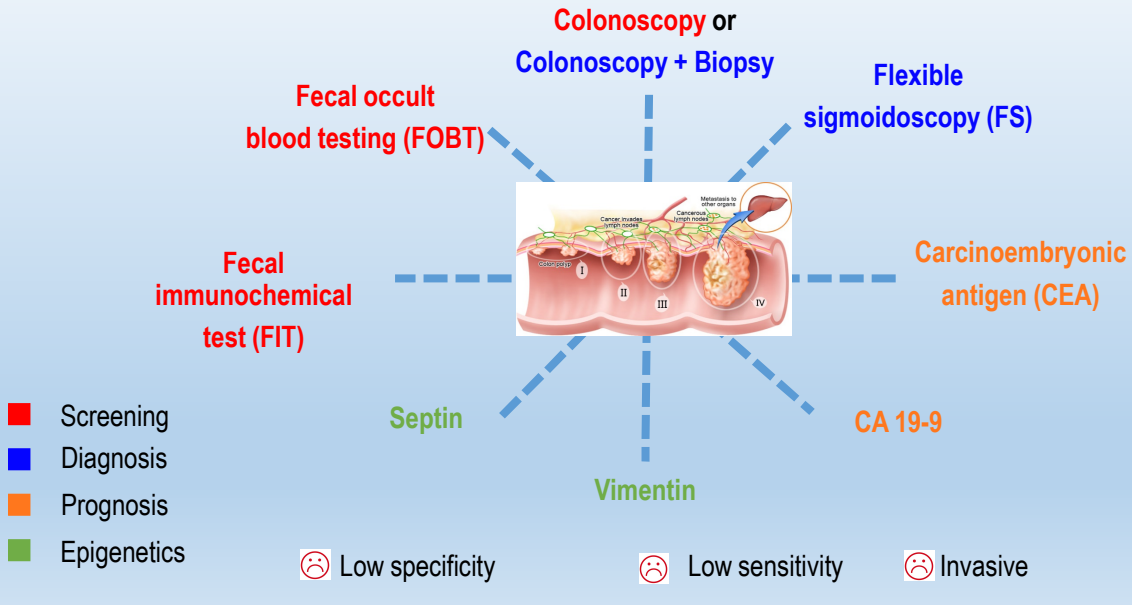
CRC is a multistage process that results from the progression of sequential accumulated **genetic** and **epigenetic alterations**



Adapted from *Terzić, et al.*, 2010

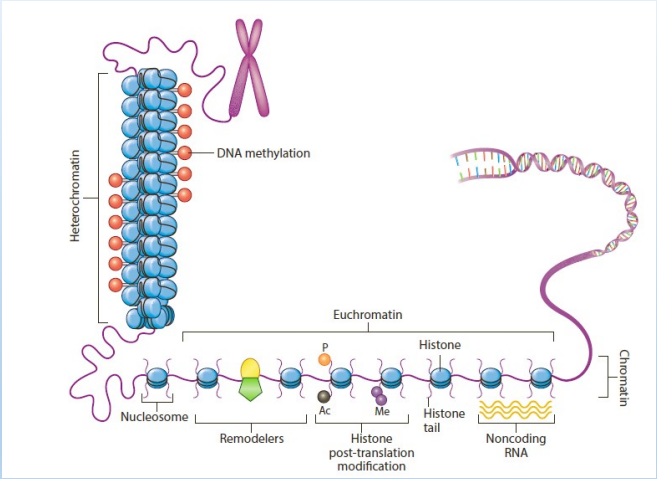
Epigenetic alterations

SCREENING, DIAGNOSIS AND PROGNOSIS OF COLORECTAL CANCER



THERE IS A NEED OF NEW TOOLS: EPIGENETIC MECHANISMS

- **DNA methylation**
- Micro RNAs
- Histone Modifications



from Ahuja et al. 2016

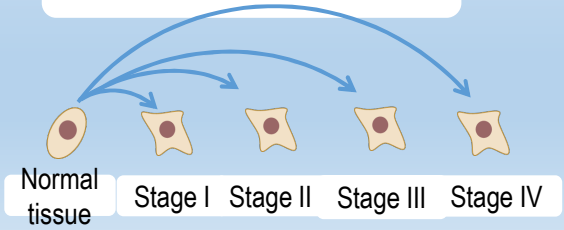
DATA ANALYSIS

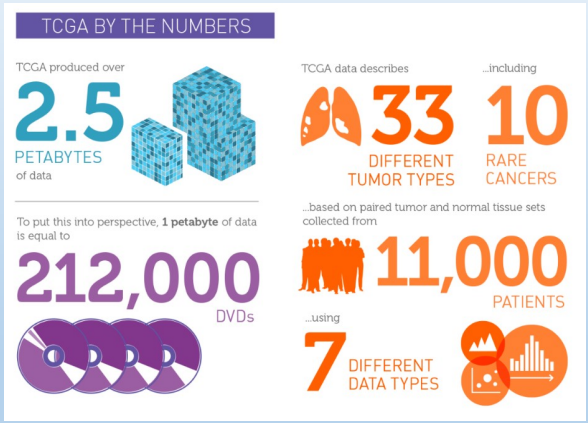
Whole genome analysis DNA methylation and gene expression

The Cancer Genome Atlas

Colon and Rectum Cohorts (n=351)

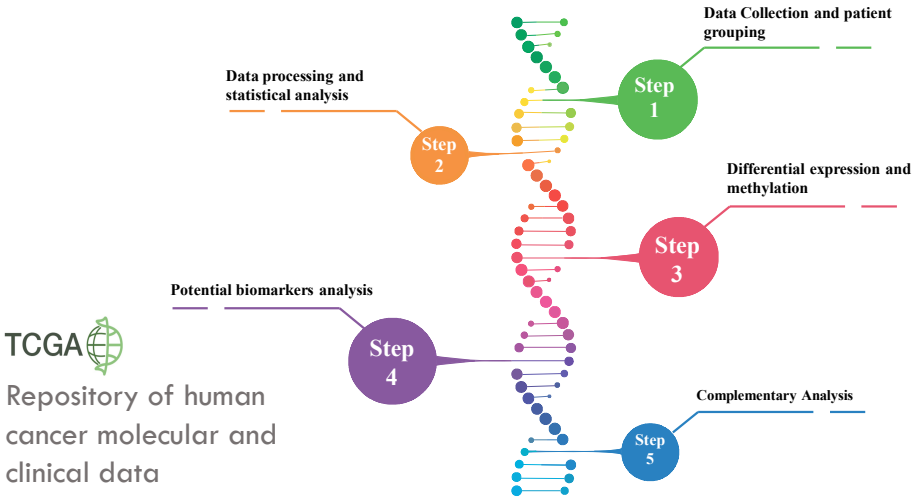
All stages of TNM classification





adapted from https://cancergenome.nih.gov/abouttcga

Study Pipeline
TCGA data extraction and analysis

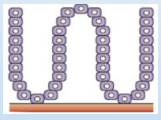


TCGA

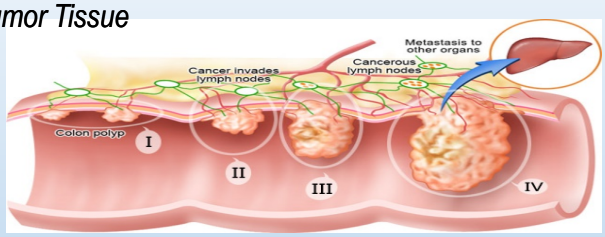
Repository of human cancer molecular and clinical data

EPIGENETIC ROADMAP OF CRC PROGRESSION

Normal Tissue



Tumor Tissue



Epigenetic changes that are maintained across CRC **PROGRESSION**

↓

IDENTIFY CANCER PATIENTS

Epigenetic changes that occur in a **SPECIFIC STAGE** of CRC

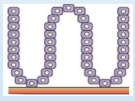
↓

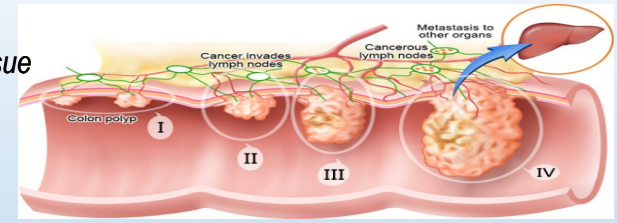
IDENTIFY THE CRC STAGE OF PATIENTS

Sara Ramalhete

EPIGENETIC ROADMAP OF CRC PROGRESSION: AN IDENTITY CARD.

Normal Tissue *Tumor Tissue*





Stage I	*Stage II*	*Stage III*	*Stage IV*
66 genes →	85 genes →	41 genes →	40 genes

61 genes

41 genes

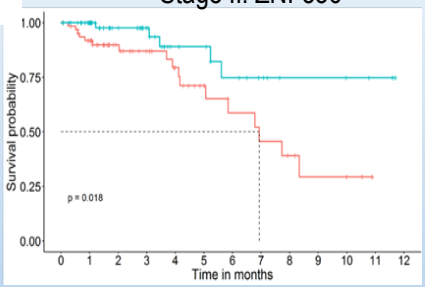
19 genes

85 genes

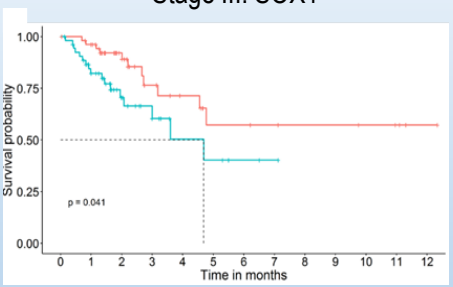
Sara Ramalhete

GENES PREDICT OVERALL SURVIVAL: KAPLAN-MEIER ANALYSIS

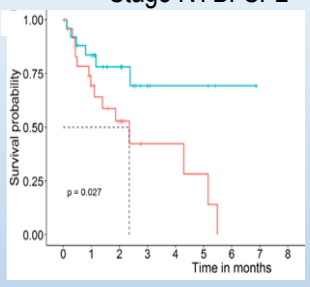
Stage II: ZNF536



Stage III: SOX1



Stage IV: BFSP2



Negative regulator of neuron differentiation

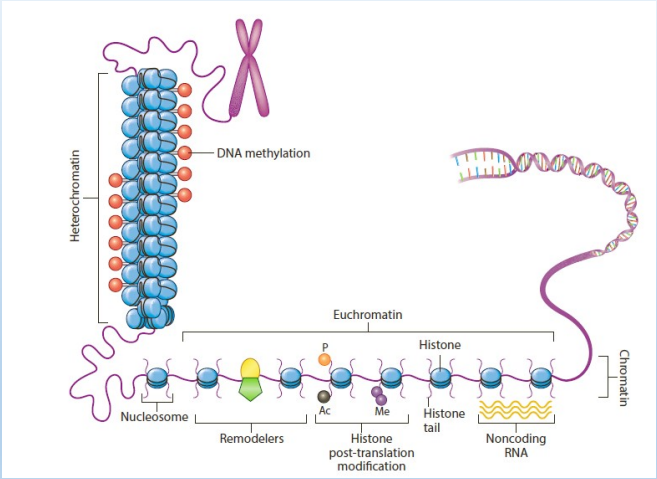
Inhibitor of cell growth and promoter of apoptosis

Codifies for a filament protein

Sara Ramalhete

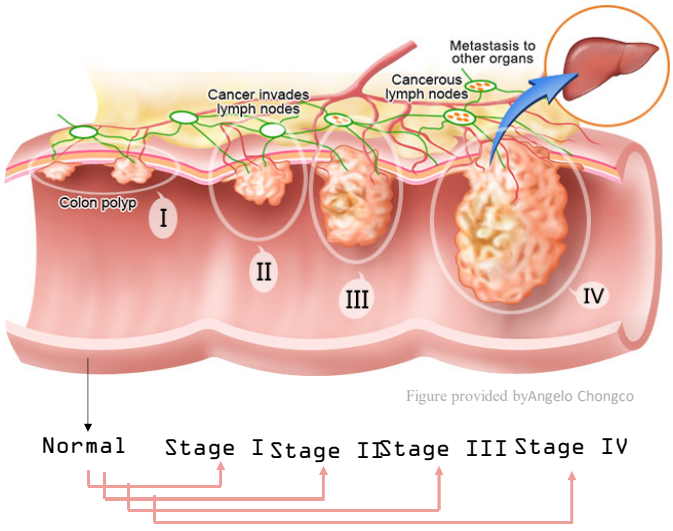
THERE IS A NEED OF NEW TOOLS: EPIGENETIC MECHANISMS

- DNA methylation
- **Micro RNAs**
- Histone Modifications



from Ahuja et al. 2016

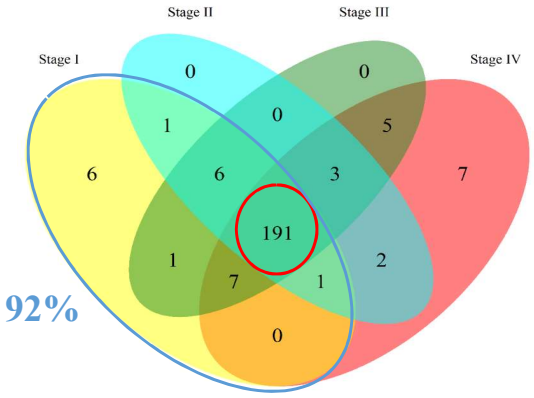
miRNA analysis



André Fonseca

MicroRNA: expression

MiRNAs expression alterations occur in an early stage



When combining the differently expressed miRNAs across all four stages

↓

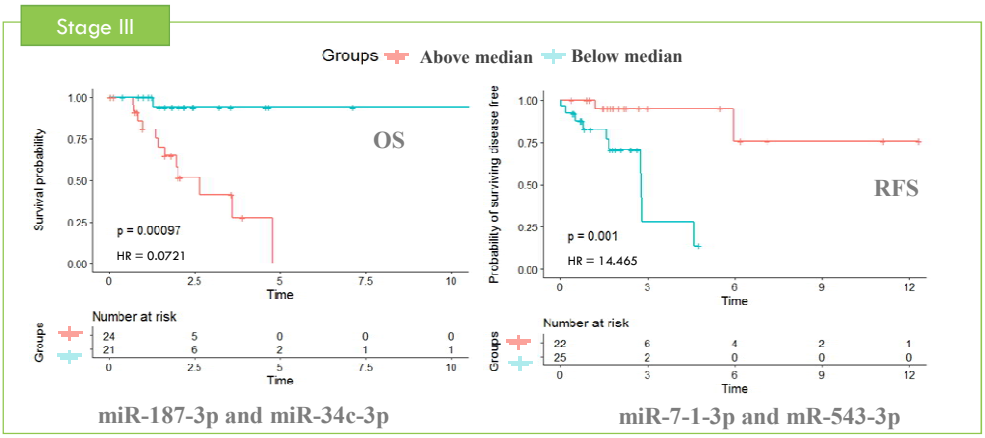
230 miRNAs

MiRNAs expression alterations occur in an early stage

André Fonseca

MicroRNA expression

Differently expressed miRNAs provide good prognostic values



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Advances and challenges in application of feedomics to improve dairy cow production and health.

Acknowledgments

Epigenetics Lab, Faro Portugal
Helder Lousada
Joana Apolonio
Sara Ramalhete
André Mestre
Allie Binnie
Ana Marreiros
Henrik Helquist
Monica Teotónio
Tatiana Lourenço

Innsbruck Medical University, Innsbruck, Austria
Helmut Klocker

Labatt Brain Tumor Research Center, Toronto
Uri Tabori
Michael D. Taylor
Cynthia Hawkins
Peter B. Dirks
Cindy Zhang
Tatiana Lipman
Aryeh Price

Princess Margaret Cancer Center, Toronto, Canada
Rob Bristow
Rob Hamilton

Max Planck Institute for Molecular Genetics, Berlin, Germany
Stefan Buerno
Michal Schweiger

Mount Sinai Hospital Toronto, Canada
Alexandre Zlotta

German Cancer Research Center (DKFZ), Heidelberg, Germany.
Holger Sueltmann