ncRNAs in cancer

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REGE
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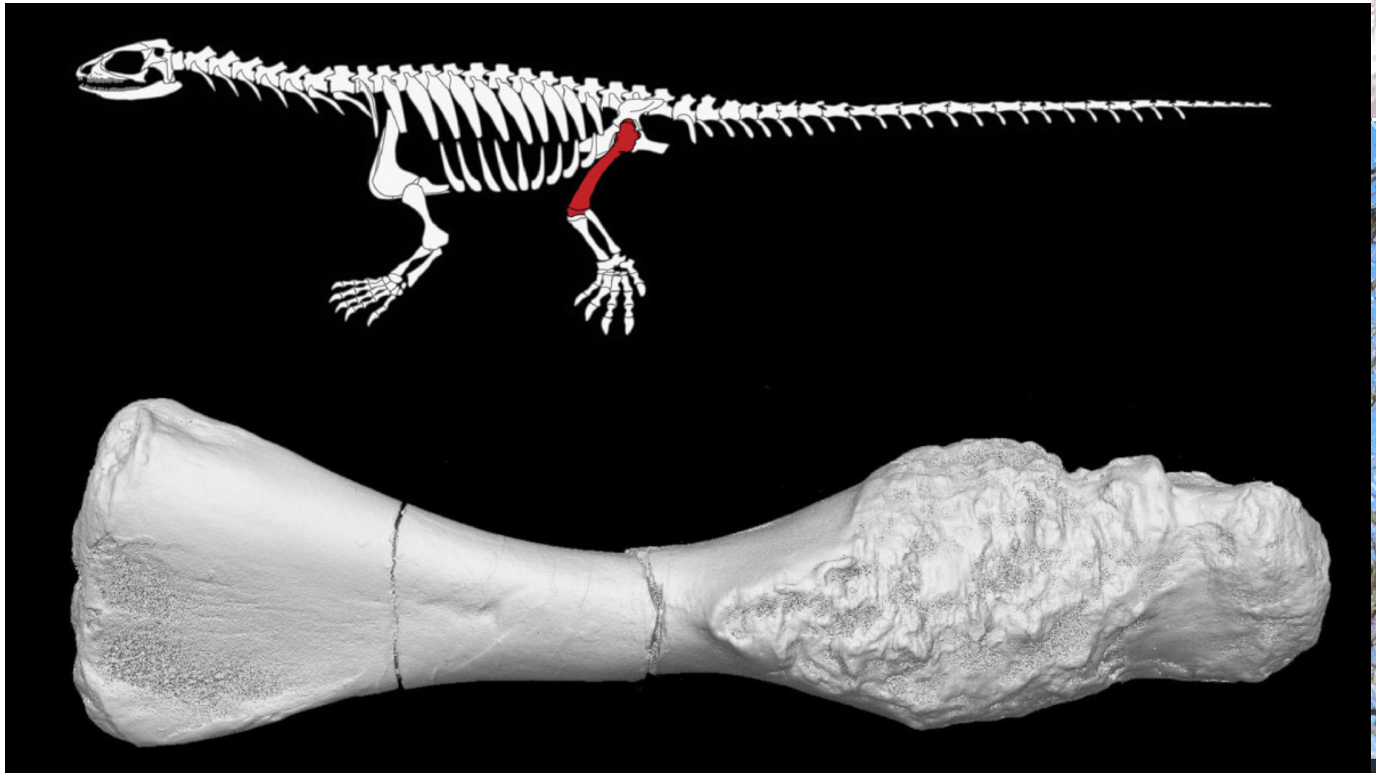
- Brief description of cancer.
- Brief reminder about ncRNAs in cancer
- Examples of ncRNAs driving diverse types of cancer
- Introduction to therapies targeting ncRNAs

Cancer is a widespread phenomena in the nature and in the history



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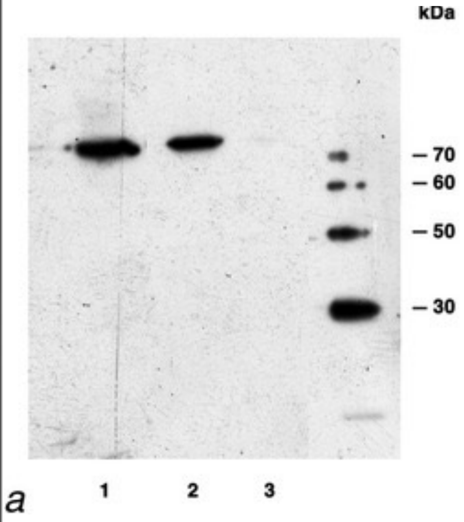
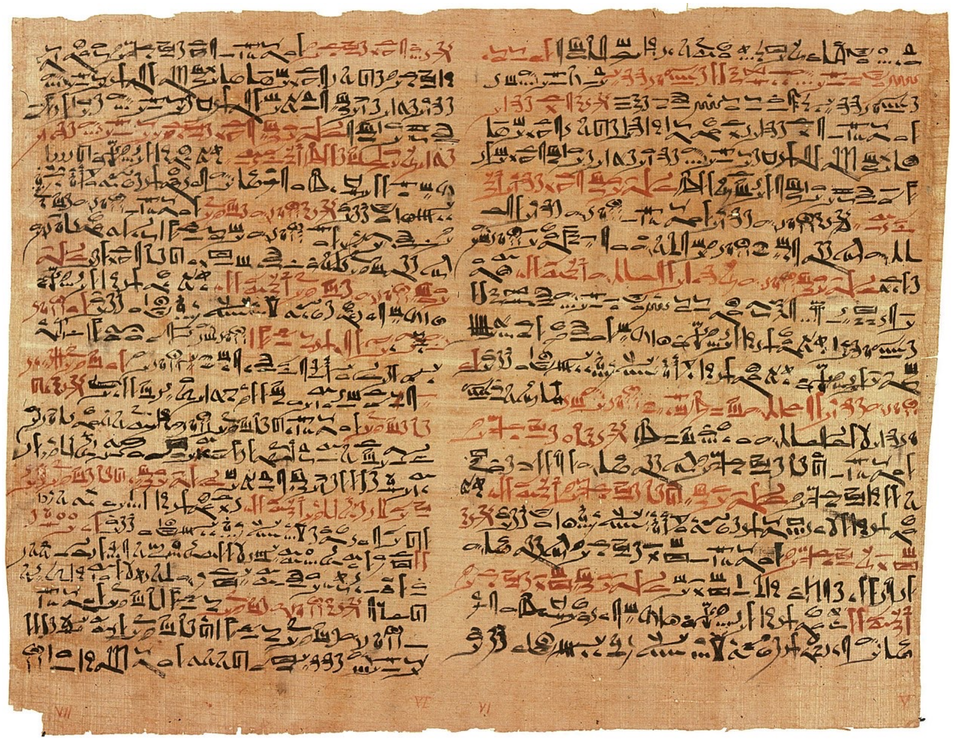
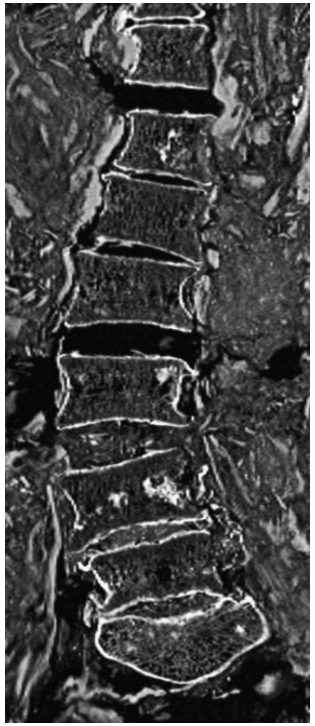


2,700-year-old Scythian king from Arzhan (Siberia, Russia). Oldest human cancer documented

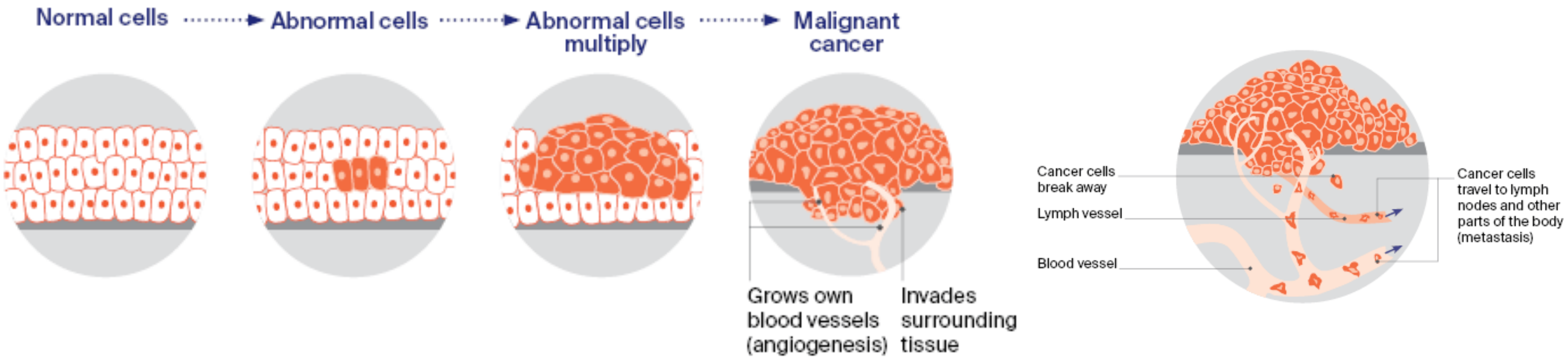


The Smith papyrus, possibly written by Imhotep the physician-architect who designed and built the step pyramid at Sakkara in the 30th century BC. First reference to cancer



Ptolemaic mummy (c. 285–30 BC)

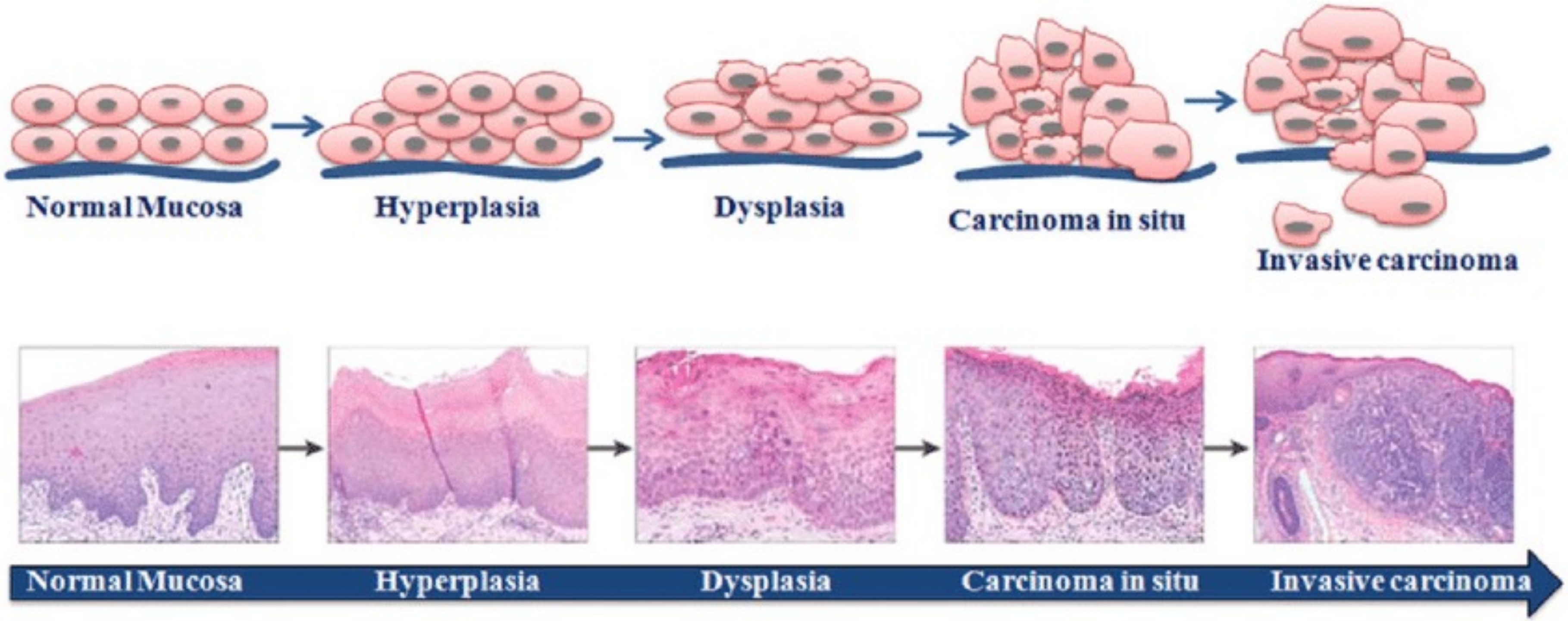
What is cancer?



Cancer is a disease of the body's cells. Normally cells grow and multiply in a controlled way, however, sometimes cells become abnormal and keep growing. Abnormal cells can form a mass called a tumour.

Cancer is the term used to describe collections of these cells, growing and potentially spreading within the body. As cancerous cells can arise from almost any type of tissue cell, cancer actually refers to about 100 different diseases

Stages of cancer



Stage 0
- Carcinoma in situ - Early form

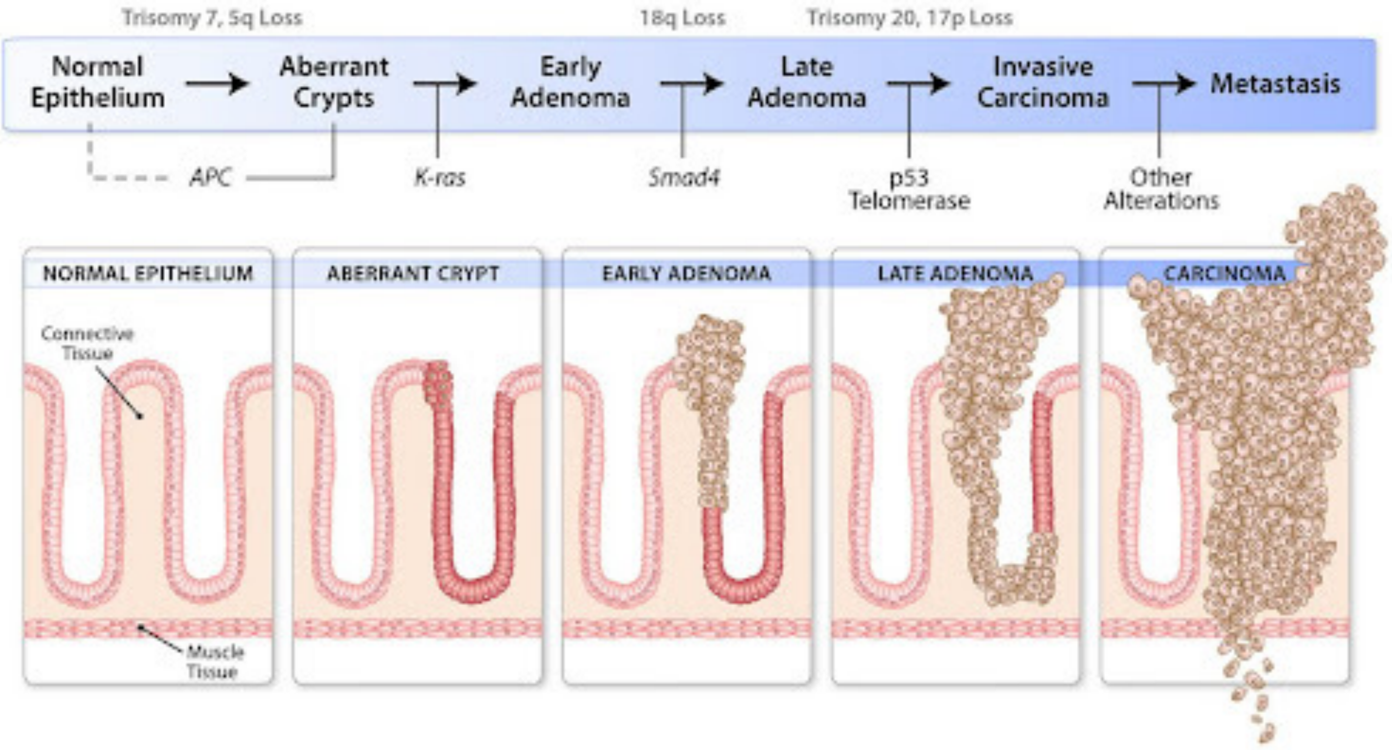
Stage I
- Localized

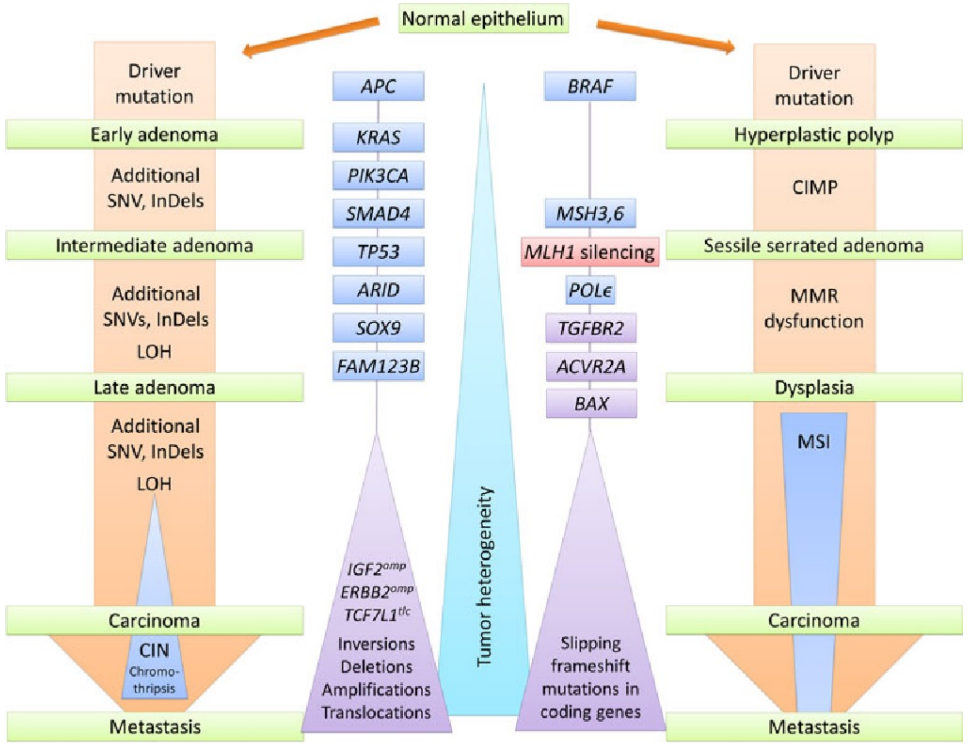
Stage II
- Early Locally Advanced

Stage III
- Late Locally Advanced

Stage IV
- Metastasized

It happen in all sort of tissues and by all sorts of molecular mechanisms





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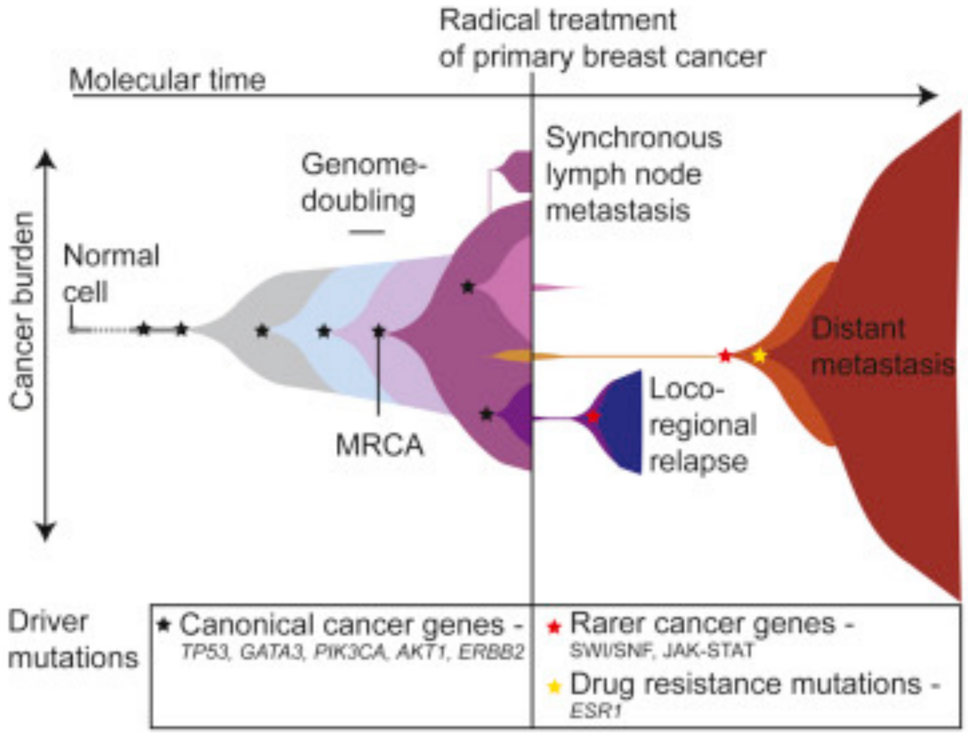
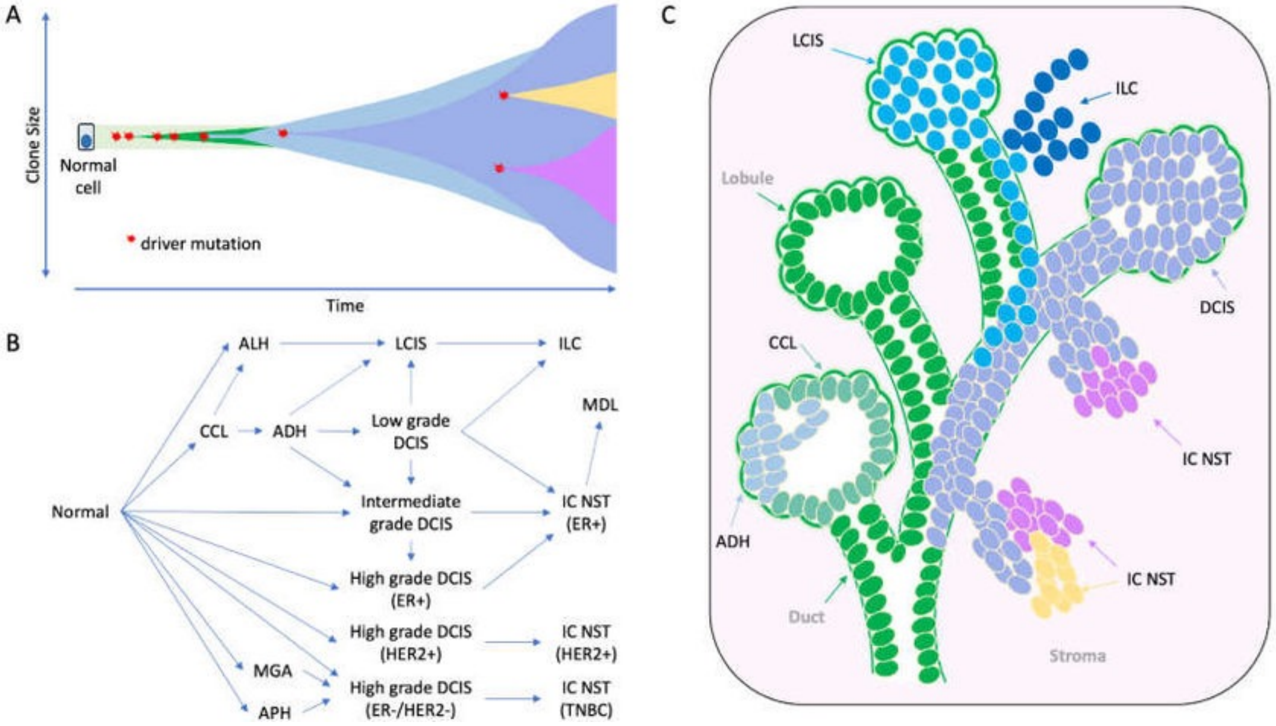
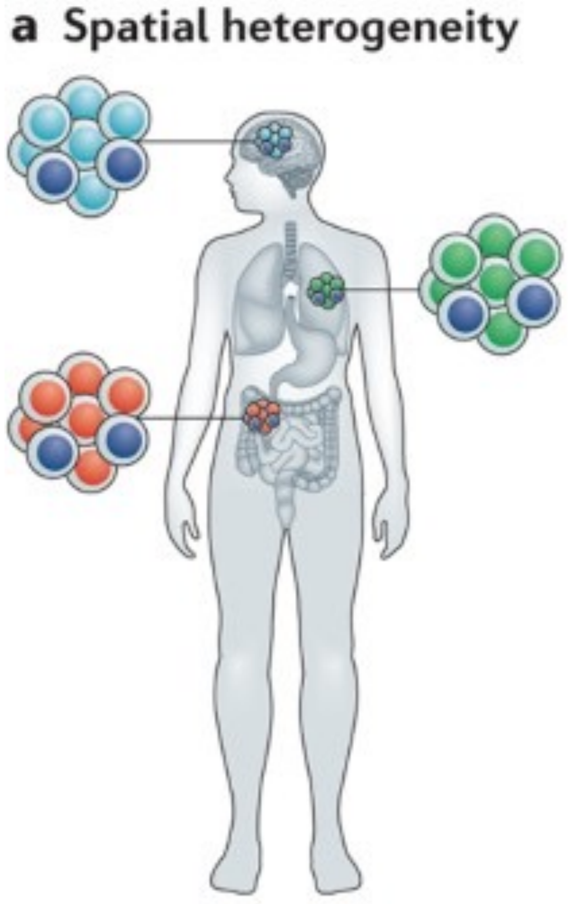
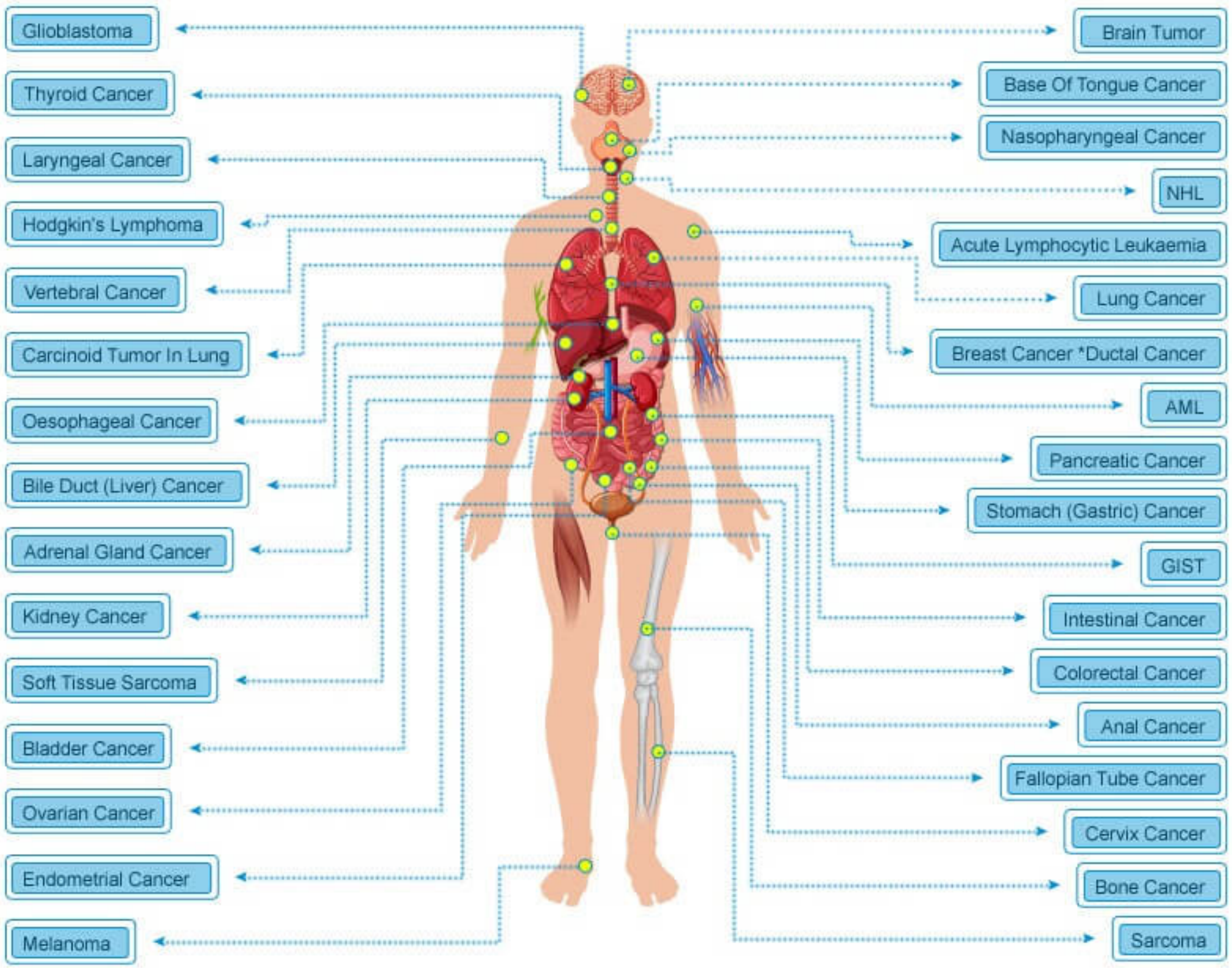


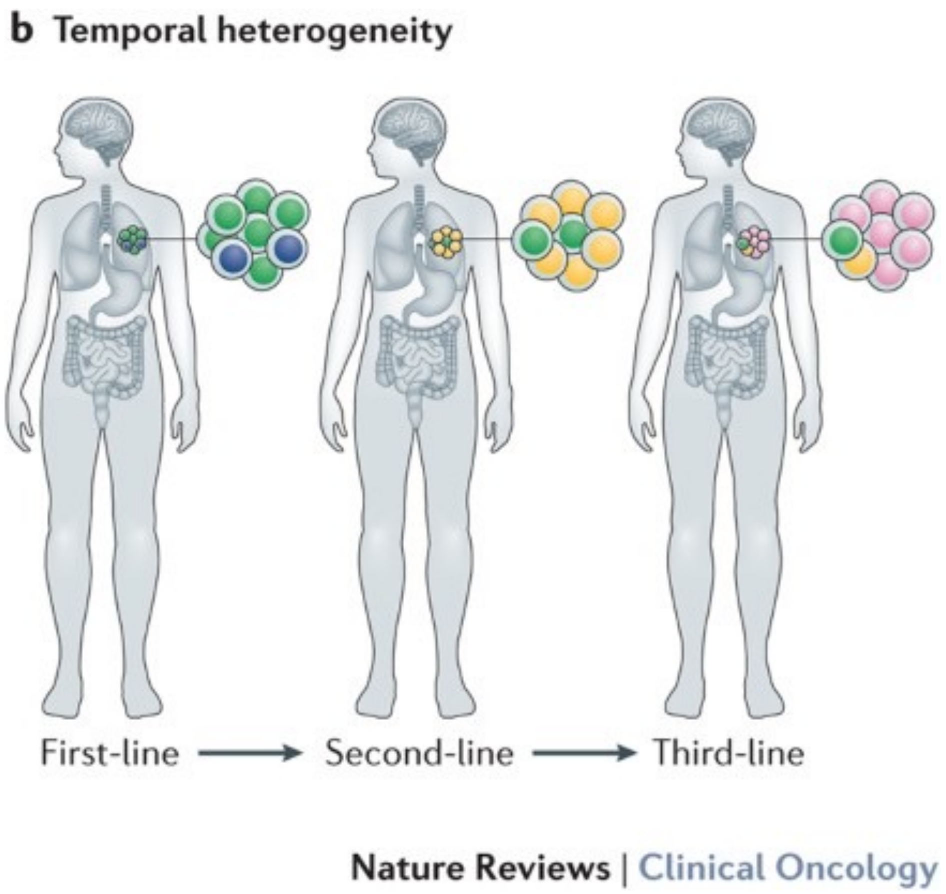


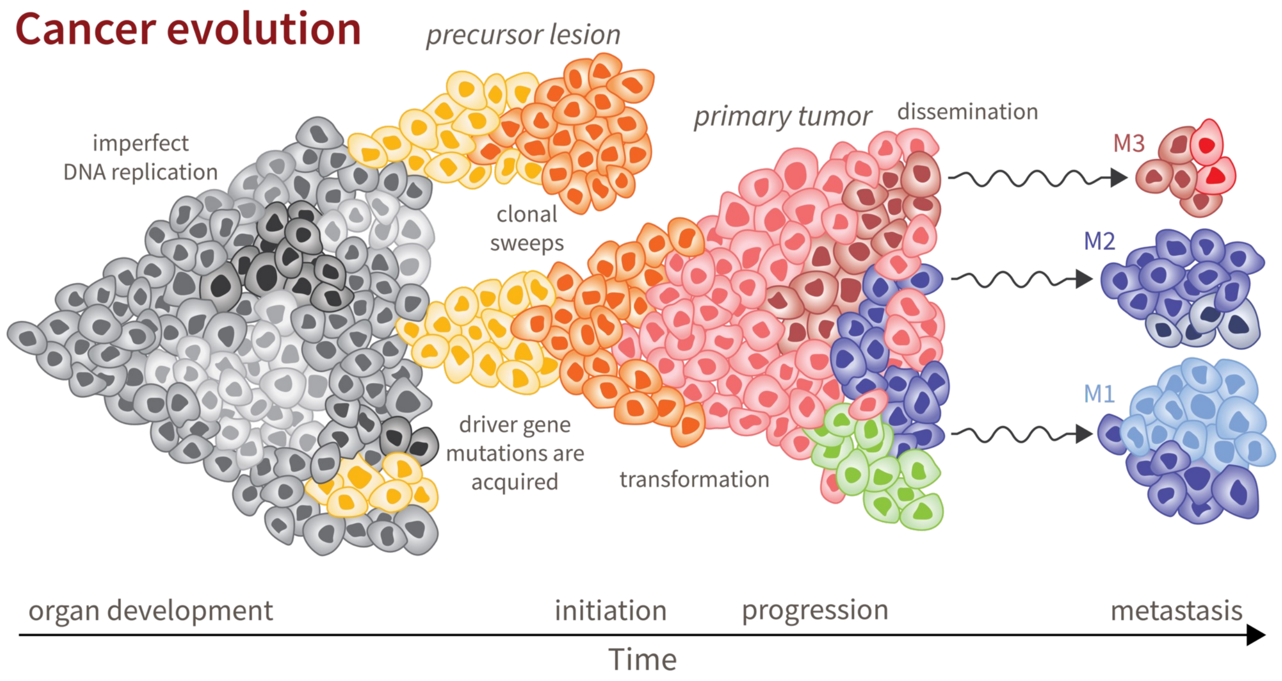
It happen in all sort of tissues and by all sorts of molecular mechanisms
Spatial heterogeneity

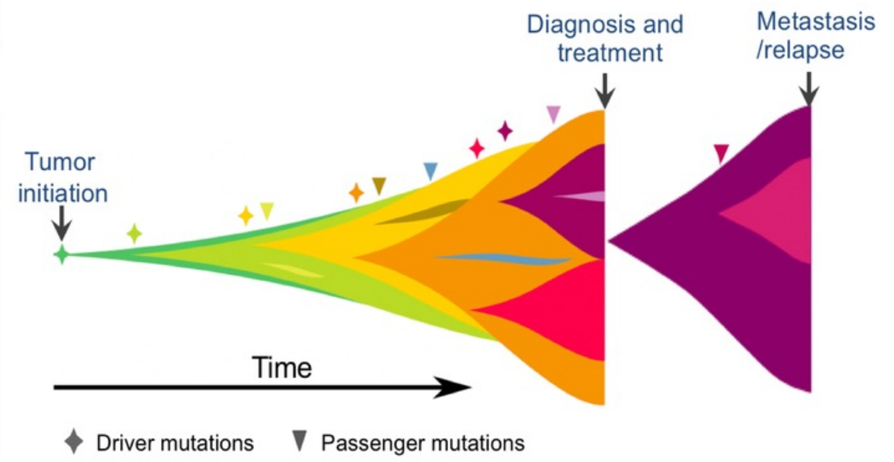




It happen in all sort of tissues and by all sorts of molecular mechanisms
Temporal heterogeneity

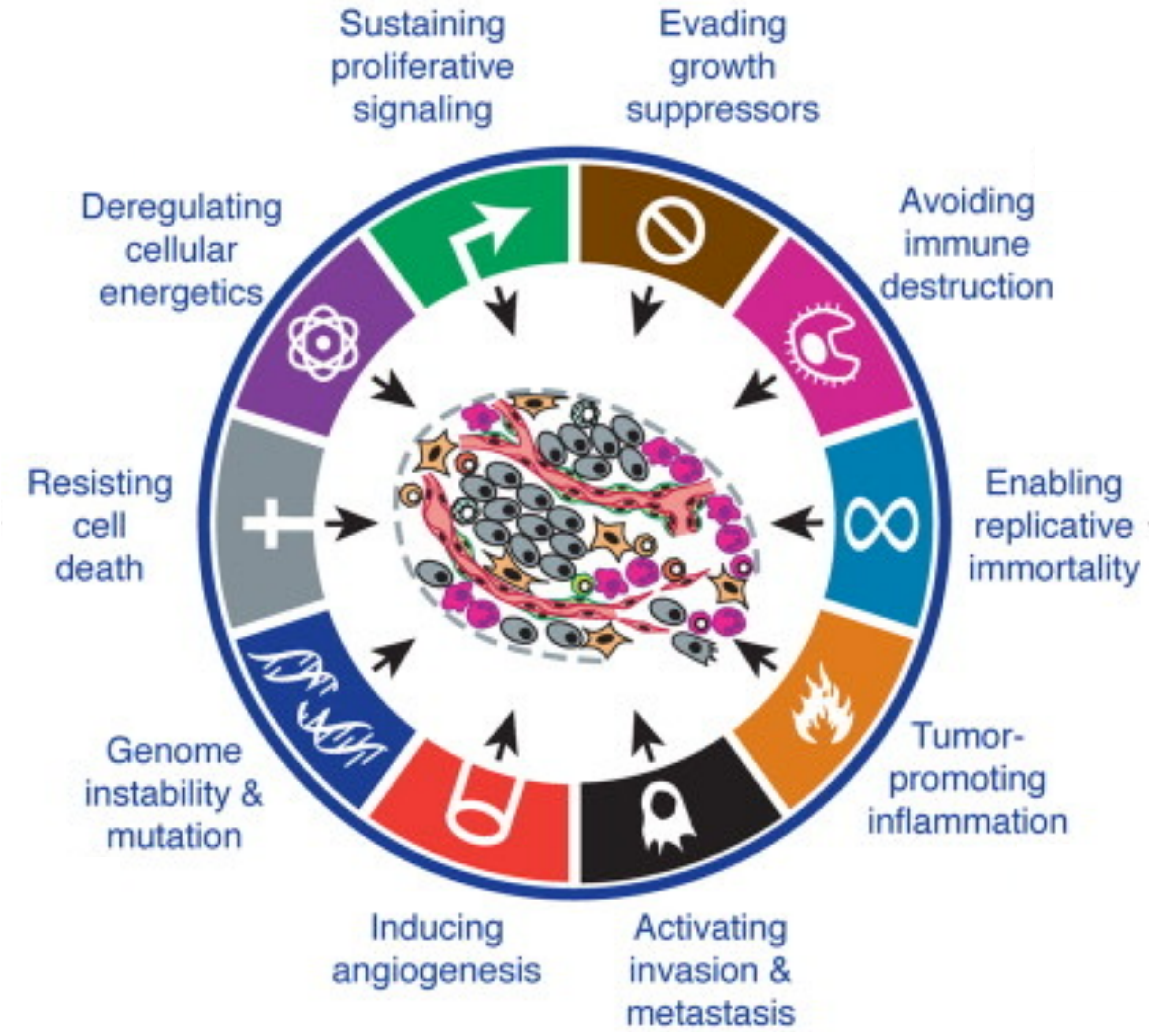






Cancer isn’t a single disease but many.
(moreover is variable *in locus* and time)

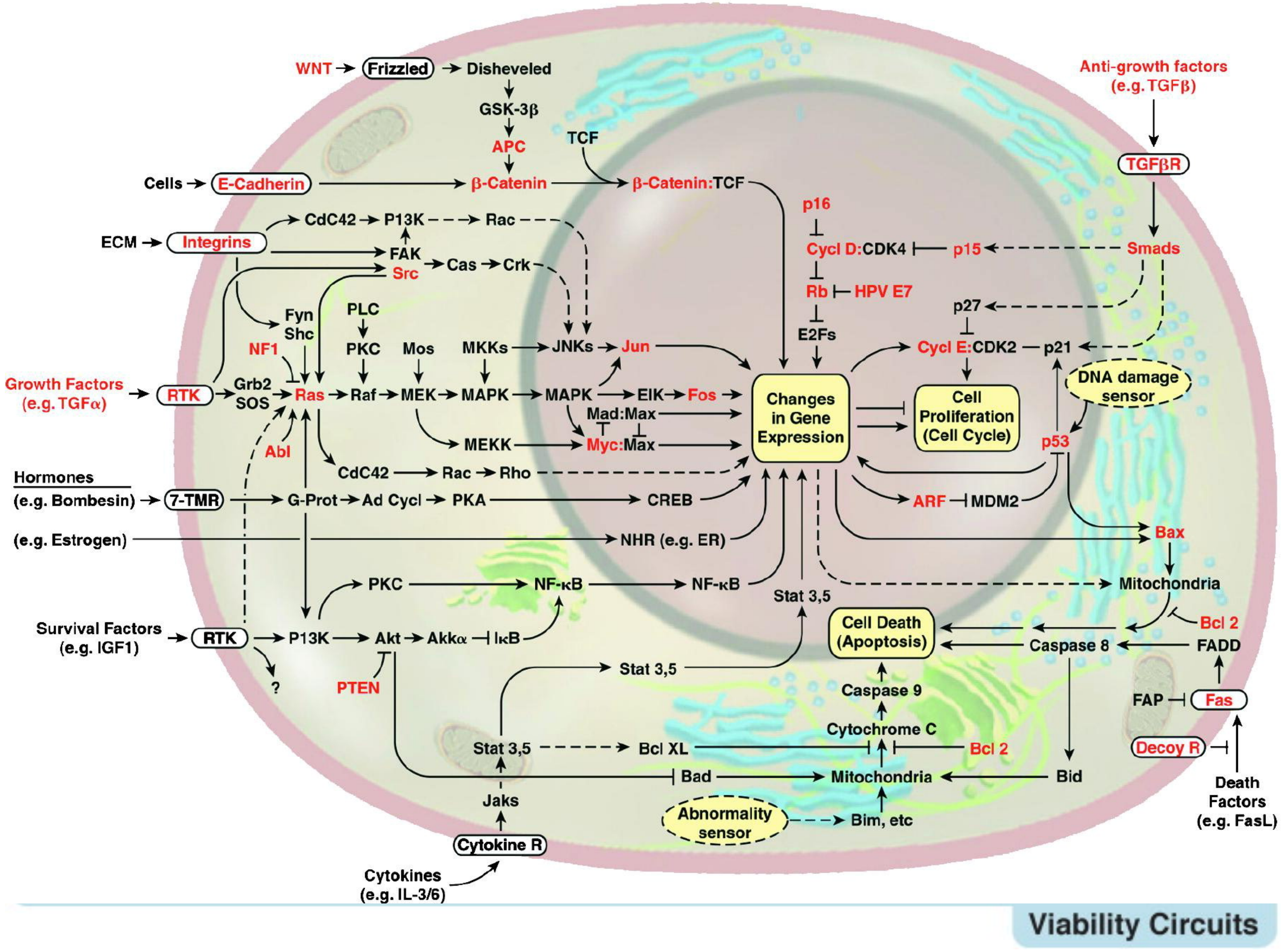
What do they have in common?



- Enabling replicative immortality
- Tumour promoting inflammation
- Activate invasion and metastasis
- Inducing angiogenesis
- Genome instability and mutation
- Cell resisting death
- Deregulating cellular energetics
- Sustaining proliferative signalling
- Evading tumour growth suppressors
- Avoid immune destruction

(Hanahan and Weinberg, 2000; Hanahan and Weinberg, 2011)

Cancer is fundamentally genetic disease than alter the cellular information flow to modify cellular homeostasis and promote cell growth



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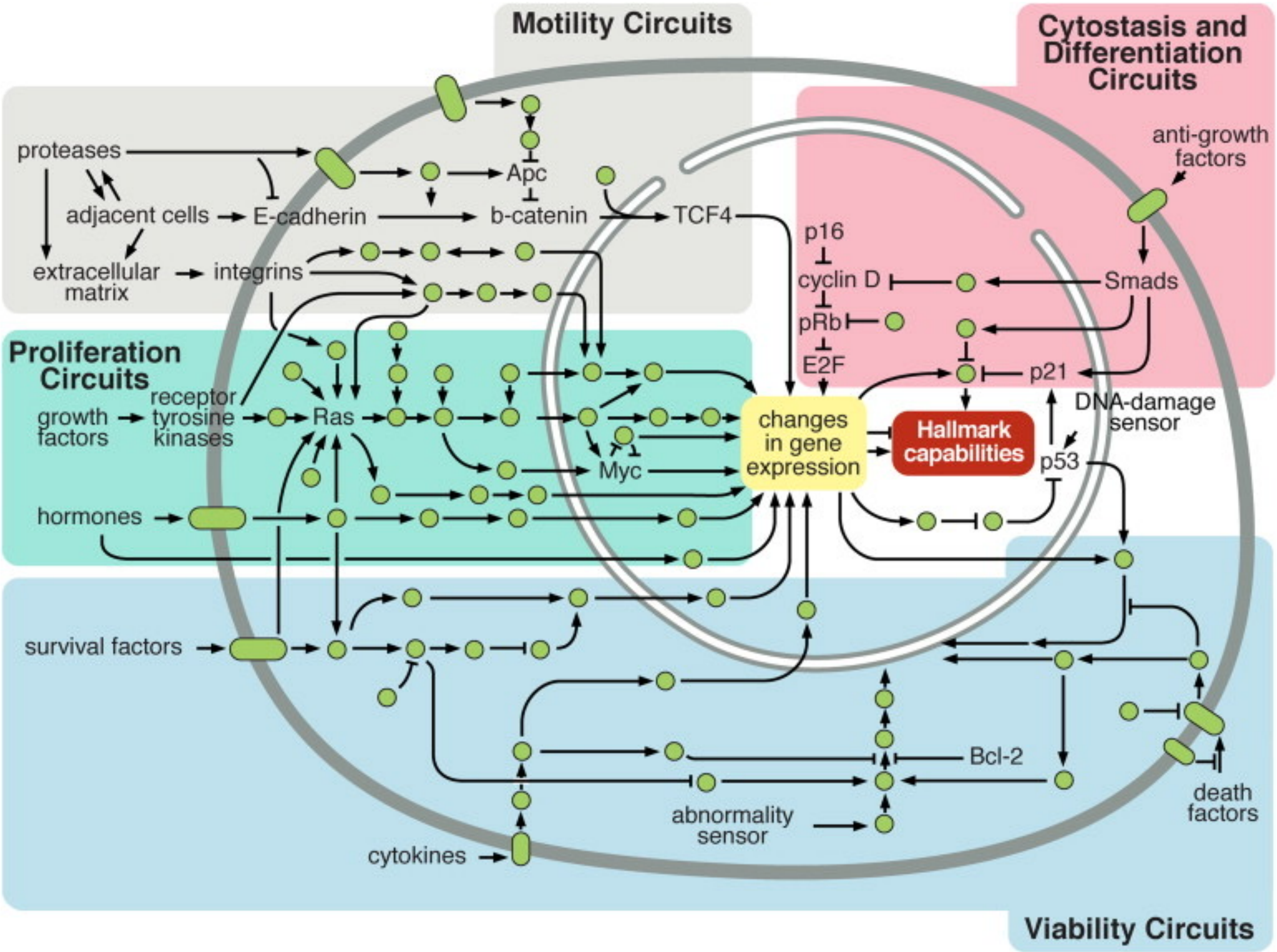

Alterations on this flow of cellular information can be caused by several factors:

- Alterations of the information in the protein coding sequence
    - BCR_ABL leukaemia, BRAFV600E melanoma
- But coding genome only counts for 2% of all transcriptome→Non coding RNA is important

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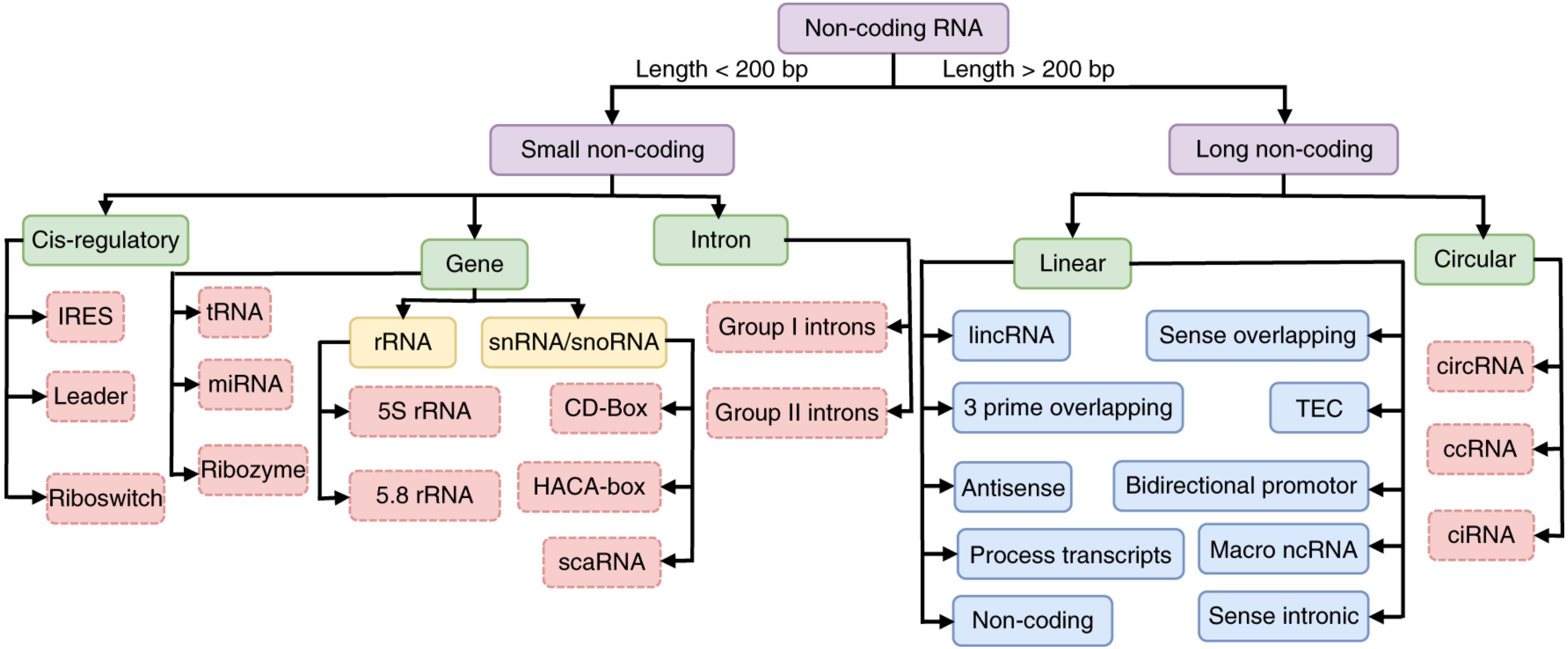
ncRNAs

A non-coding RNA (ncRNA) is an RNA molecule that is not translated into a protein.



It has been seen during last years "aberrant" non coding RNA are associated with some cancer types

LncRNAs Associated with Common Cancer Genomic Alterations			
LncRNA	Cancer Type	Genomic Alteration	References
PVT1	Colorectal	8q24 amplification	(Tseng et al., 2014)
PCAT-1	Prostate	8q24 SNPs	(Eeles et al., 2008; Prensner et al., 2011)
CCAT2	Colorectal	8q24 SNPs	(Ling et al., 2013b; Tomlinson et al., 2007)
PTCSC3	Thyroid	rs944289	(Jendrzejewski et al., 2012)
HULC	Hepatocellular	rs7763881	(Liu et al., 2012)
ANRIL	Various	9p21.3 SNPs	(Pasmant et al., 2011)
TERC	Oral cavity	3q26 amplification	(Dorji et al., 2015)
GAS5	Hepatocellular	rs145204276	(Tao et al., 2015)

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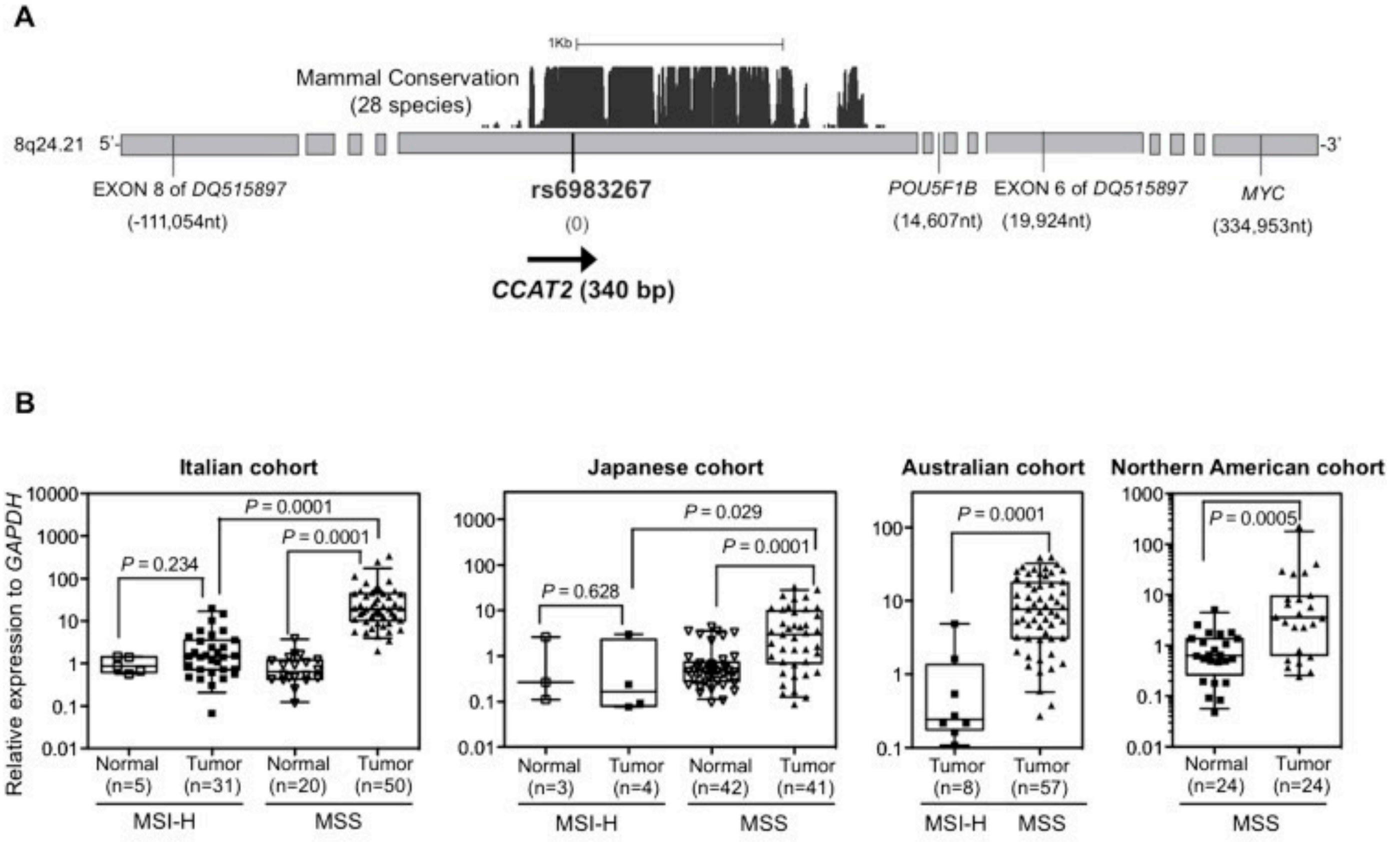
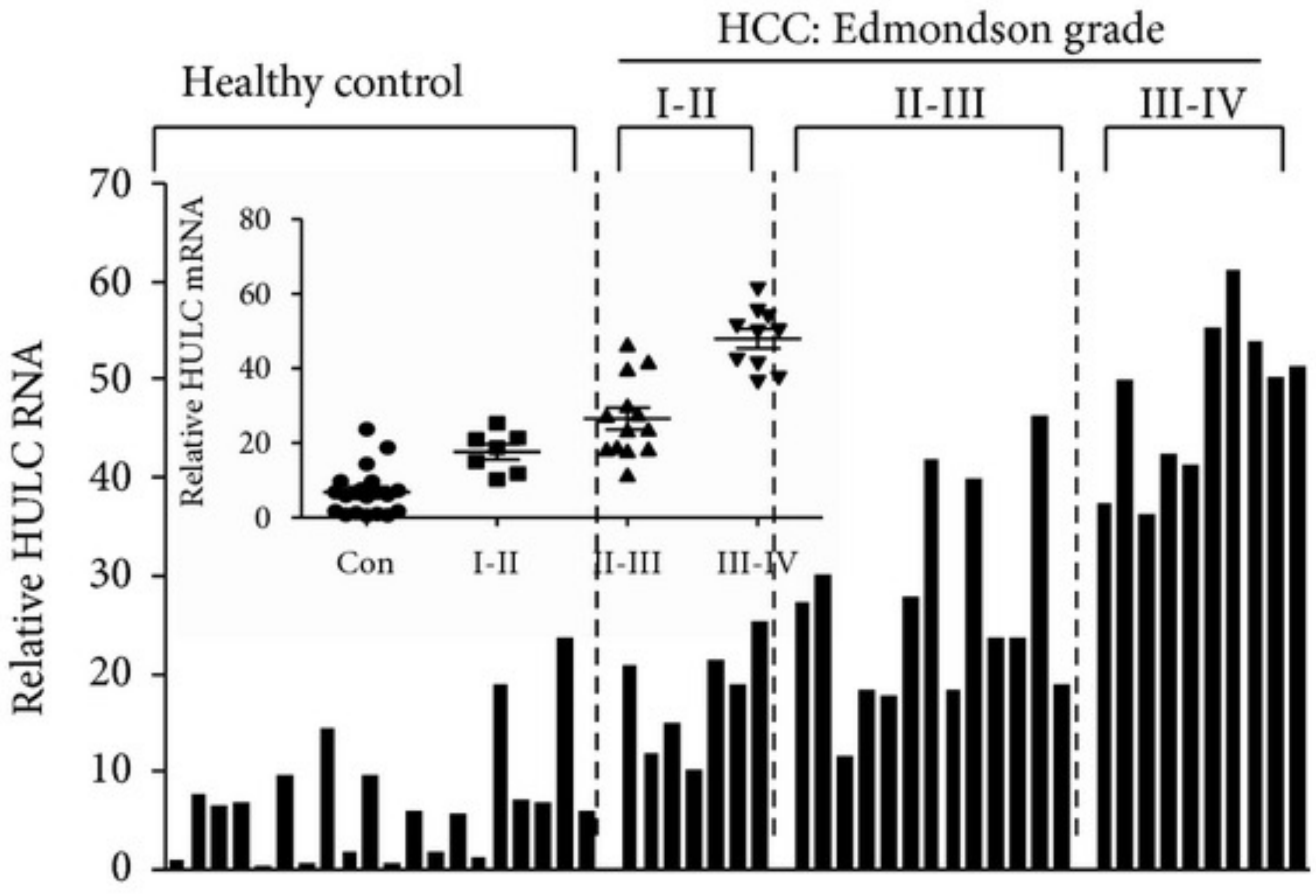


CCAT2 is associated with colorectal carcinoma

In fact, as non coding RNAs can be use to monitor the stage of the cancer: Biomarkers

LncRNAs in Cancer Diagnosis and Monitoring			
LncRNA	Cancer Type	Bioavailability of LncRNA	References
H19	Gastric	Blood	(Zhou et al., 2015)
HULC	Hepatocellular	Blood	(Xie et al., 2013)
AA174084	Gastric	Gastric secretions	(Shao et al., 2014)
PCA3	Prostate	Urine	(Bussemakers et al., 1999)
SeCATs	Sezary	Tumor	(Lee et al., 2012)
SPRY4-IT1	Melanoma	Tumor	(Khaitan et al., 2011)

In fact, as non coding RNAs can be use to monitor the stage of the cancer



HULC RNA levels in blood increase in late stages of hepatocellular carcinoma

Or than some ncRNAs are associated with which development the tumour will have or how will they respond to the drugs

Prognostic LncRNAs			
LncRNA	Cancer Type	Prognostic Information	References
FAL1	Ovarian	Poor prognosis	(Hu et al., 2014a)
HOTAIR	Breast	Increased risk of metastasis	(Gupta et al., 2010)
HOTTIP	Hepatocellular	Increased risk of progression	(Quagliata et al., 2014)
MEG3	Meningioma	Associated with tumor grade and risk of progression	(Zhang et al., 2010)
NBAT-1	Neuroblastoma	Good prognosis	(Pandey et al., 2014)
NKILA	Breast	Decreased risk of metastasis	(Liu et al., 2015)
SCHLAP1	Prostate	Increased risk of metastasis	(Prensner et al., 2014b)
LncRNAs Predicting Therapeutic Responsiveness			
LncRNA	Cancer Type	Therapeutic Agent	References
CCAT1	Colorectal	BET inhibitors	(McCleland et al., 2016)
HOTAIR	Ovarian	Platinum chemotherapies	(Teschendorff et al., 2015)

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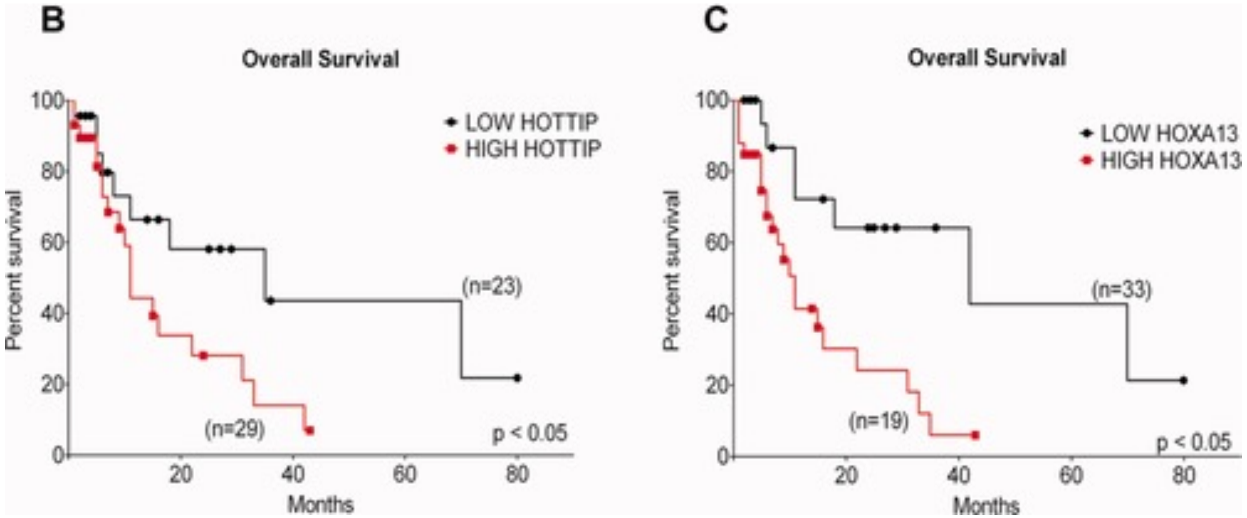
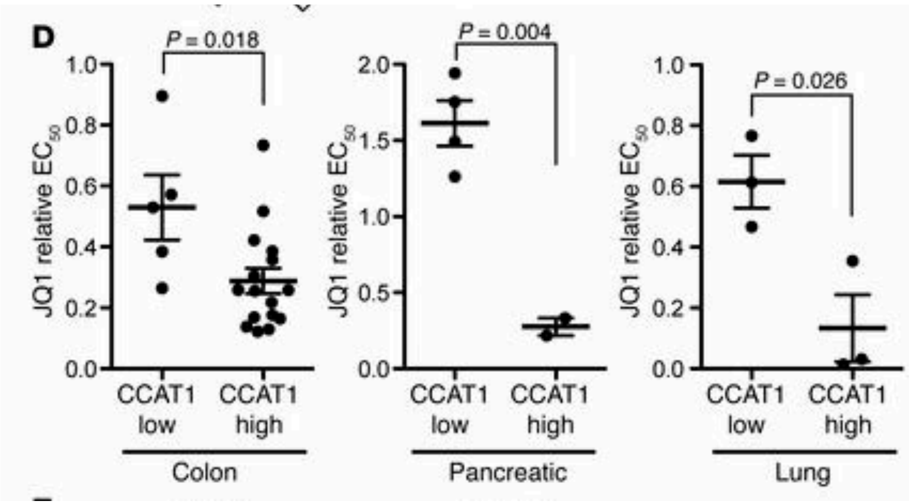


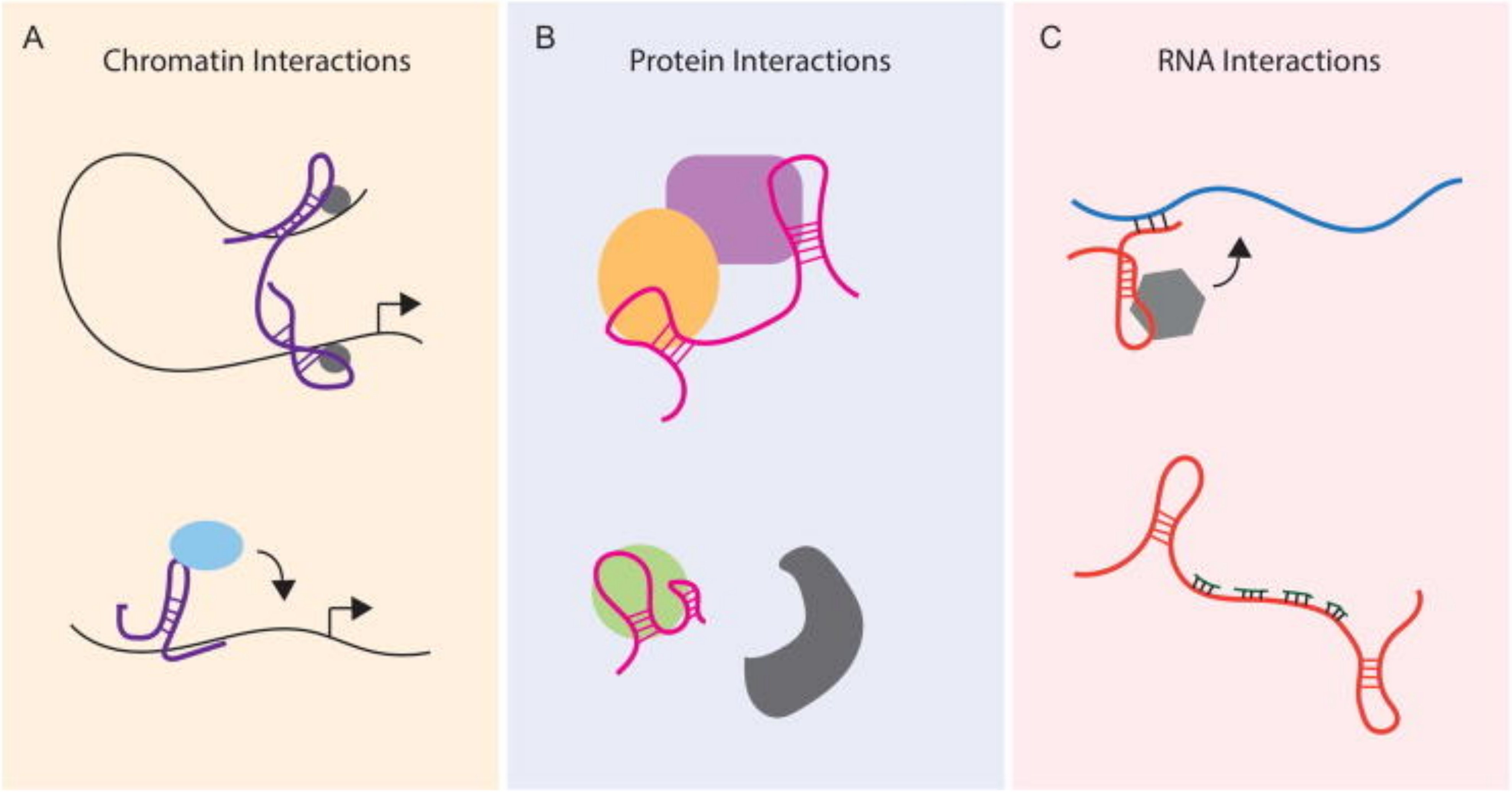


HOTTIP/HOXA13 expression is associated with poor prognosis in hepatocellular carcinoma

CCAT1 expression is linked with enhanced chemotherapy (to JQ1, BET inhibitors family chemotherapeutics) resistance in several cancers.

ncRNAs

Can regulate effector genes using different pathways



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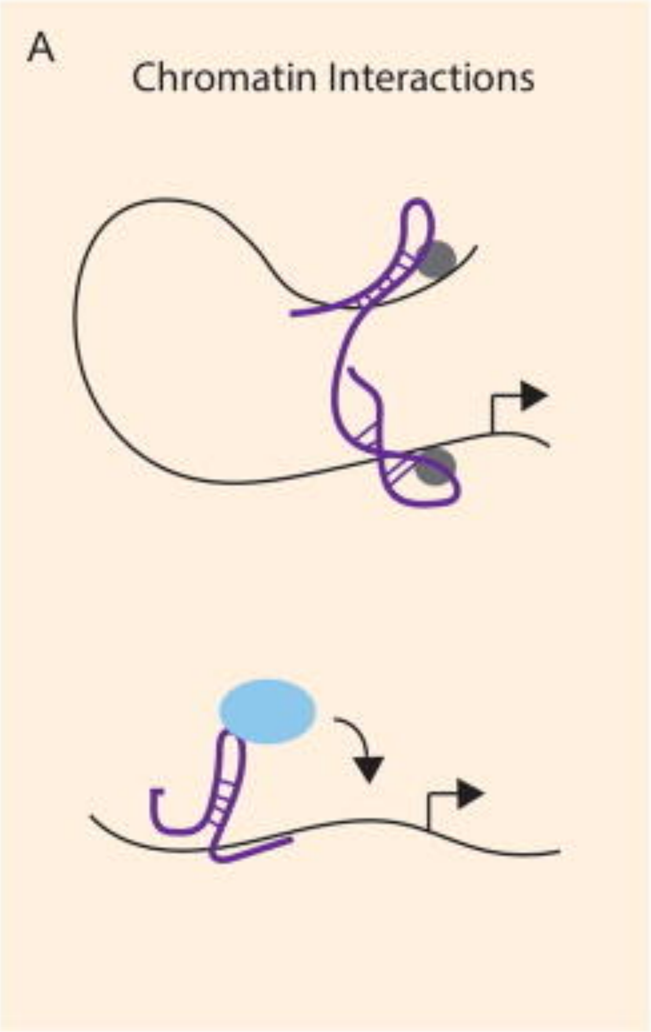
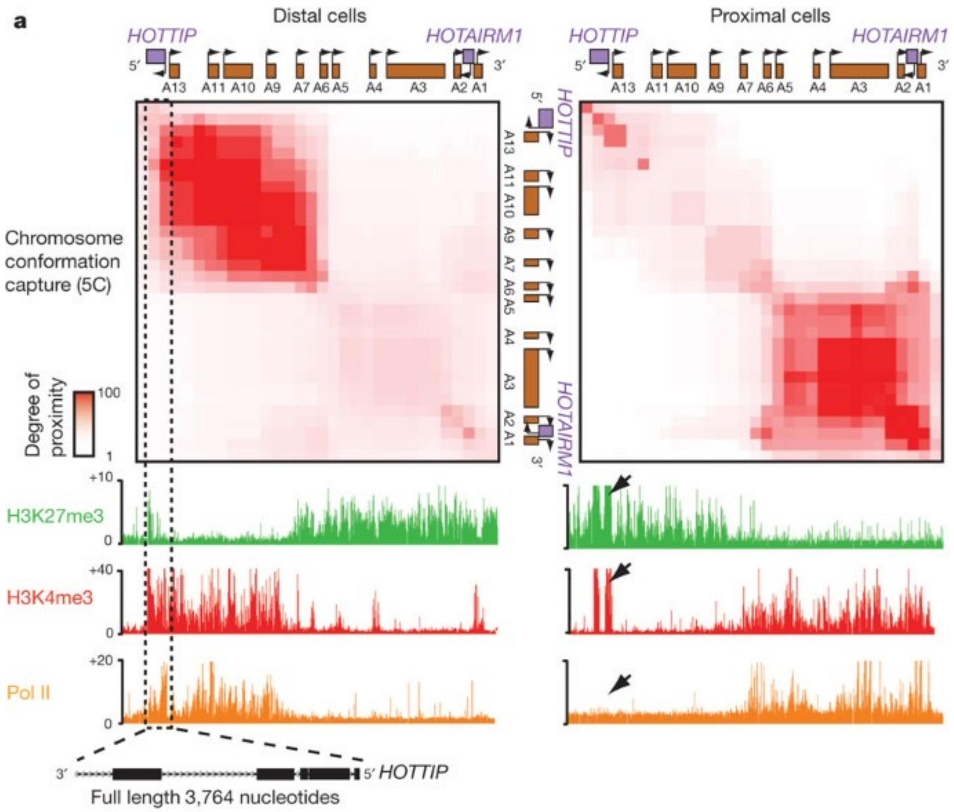
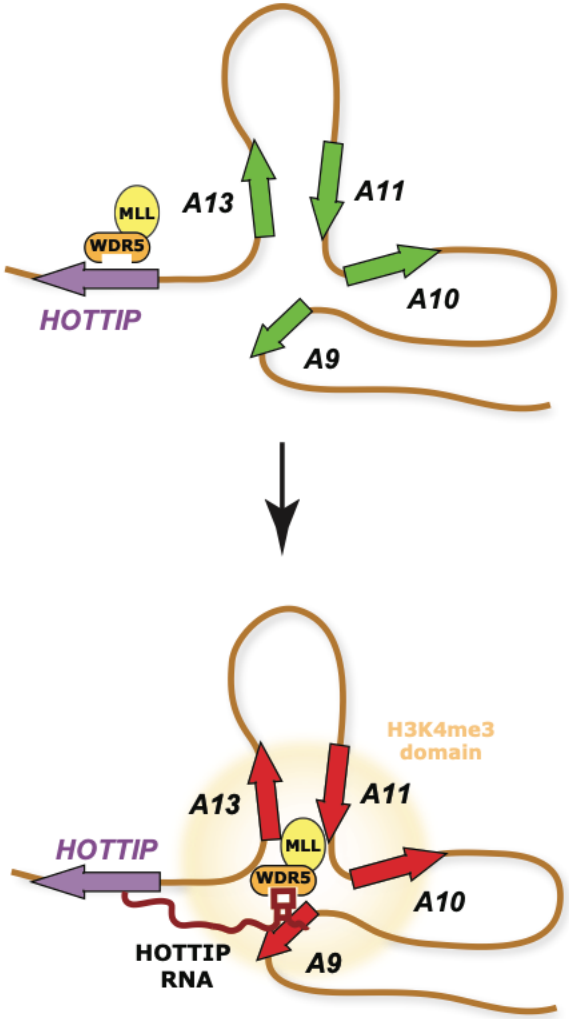




HOTTIP expression activates neighbouring genes.



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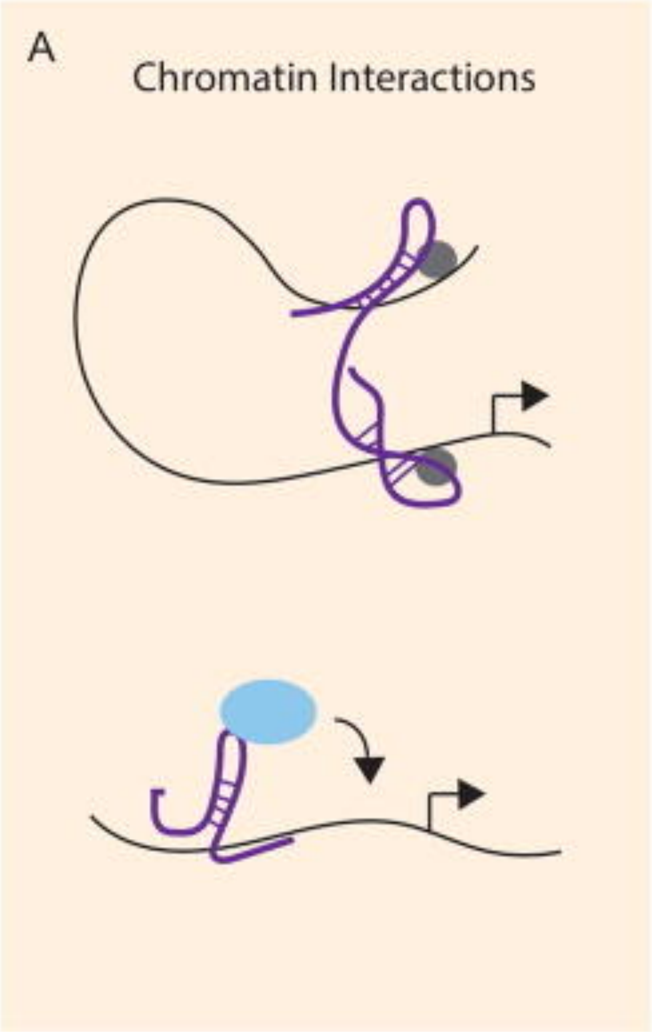
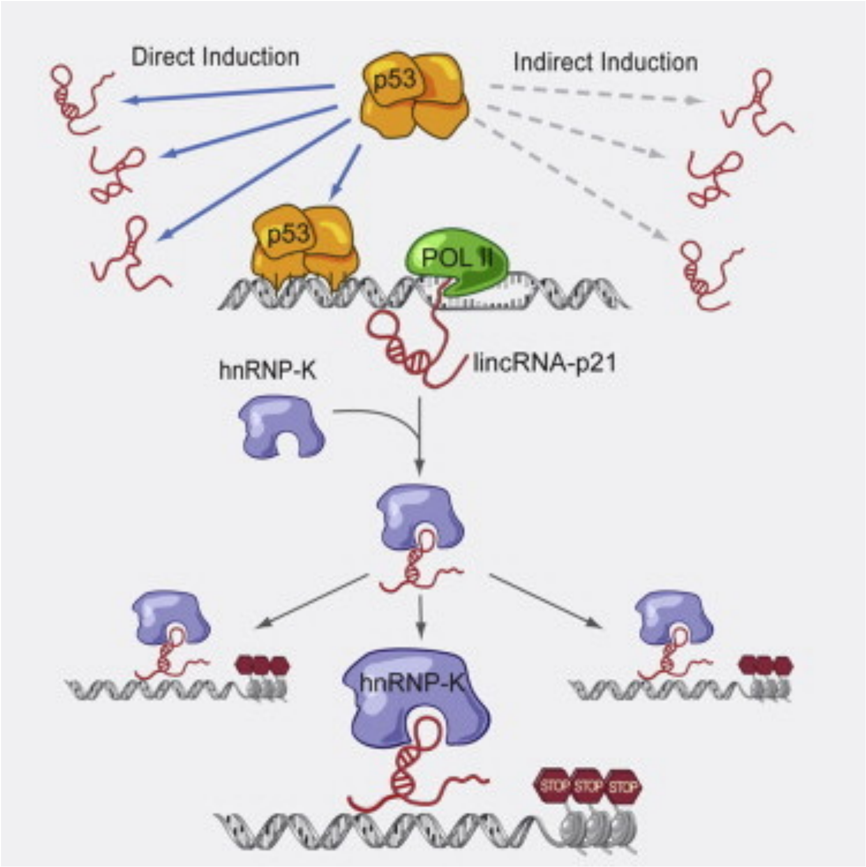
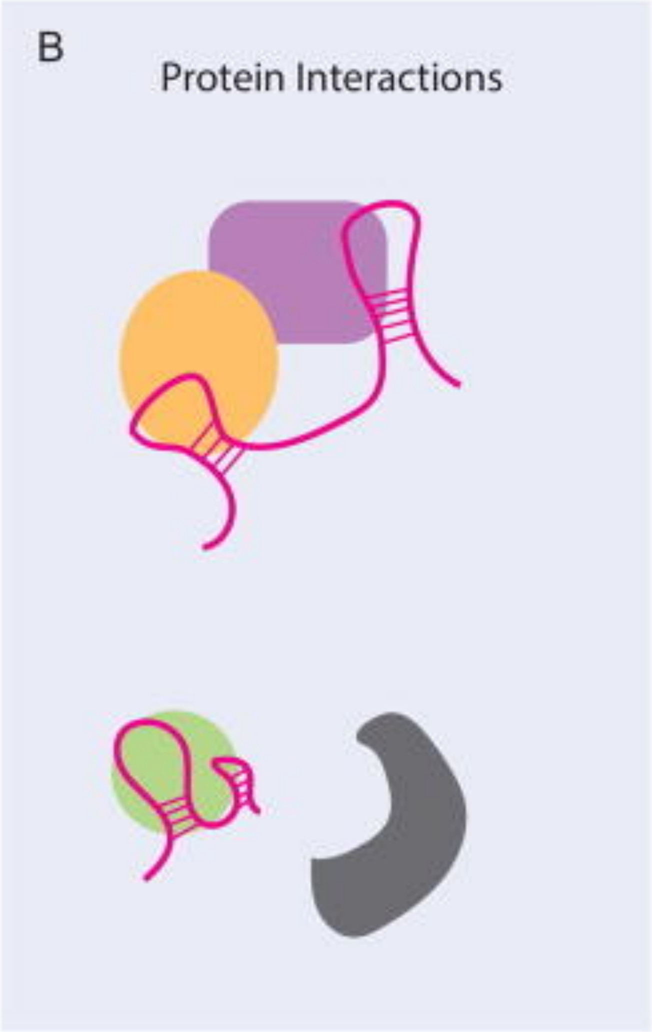
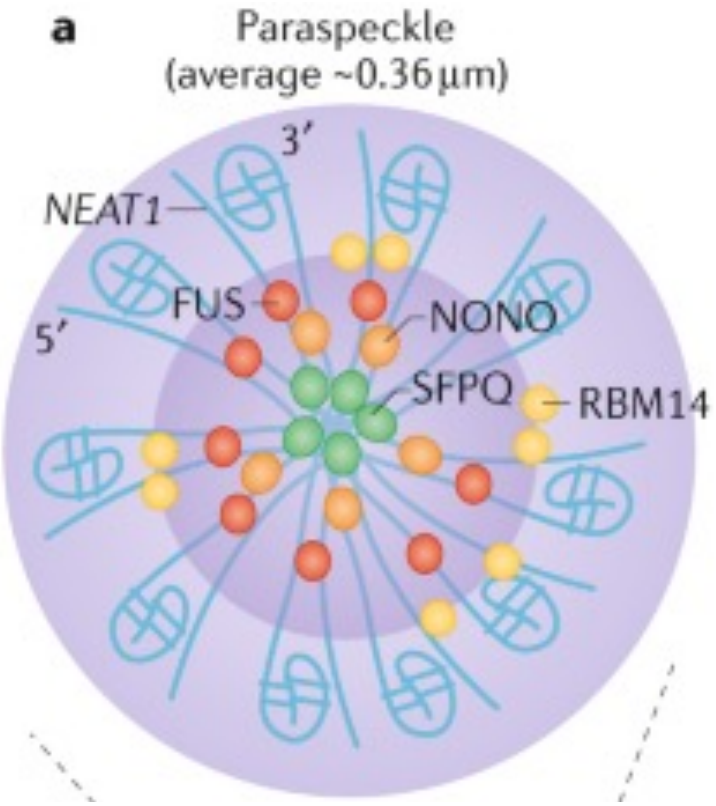




p53 response to DNA damage includes several ncRNAs mediating gene repression

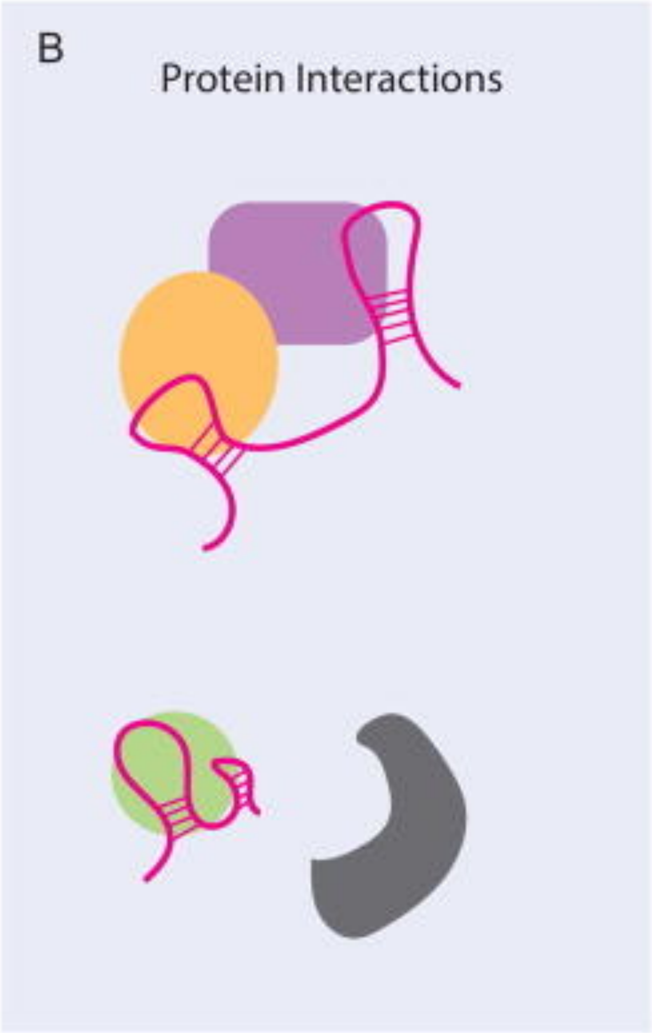
ncRNAs
Can regulate effector genes using different pathways

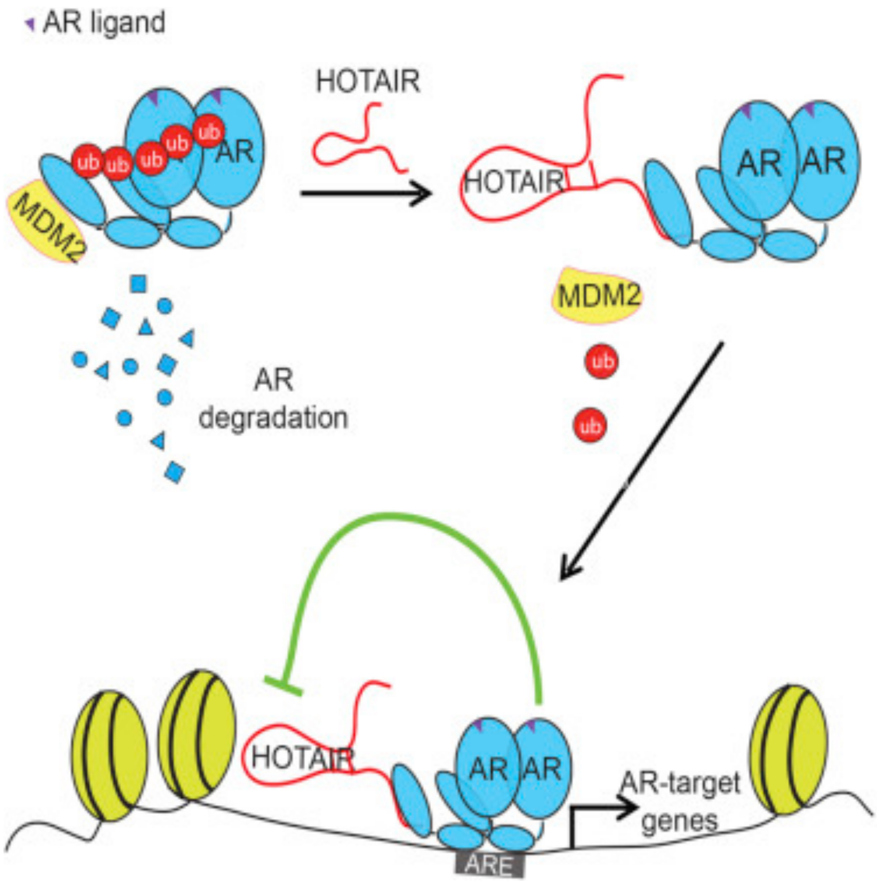




In Paraspeckles, RNA NEAT1 is necessary for the correct assembly of the proteins.

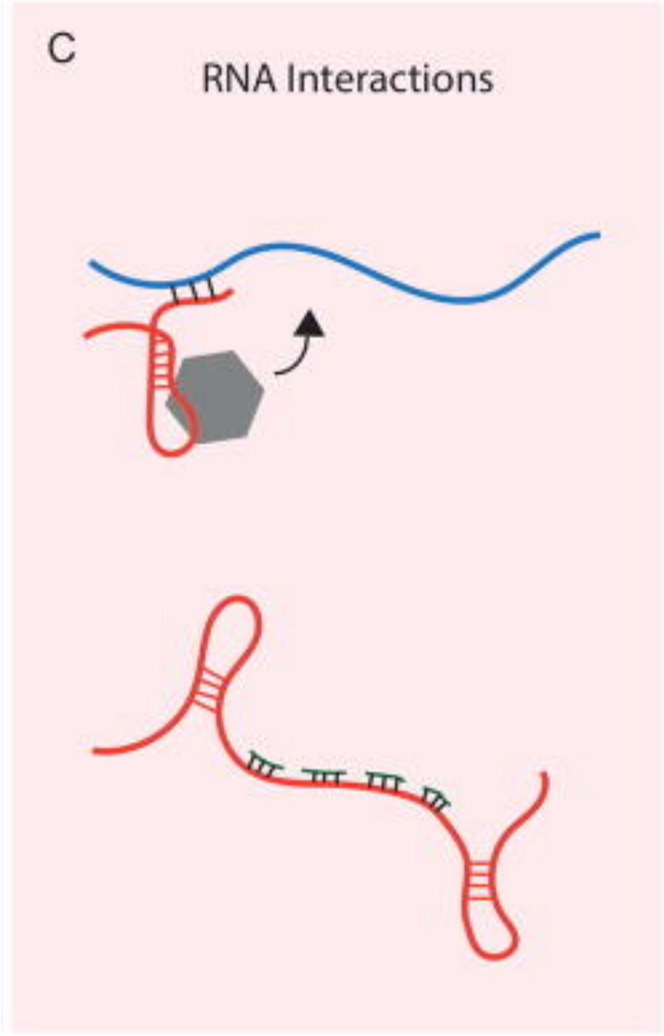
ncRNAs
Can regulate effector genes using different pathways

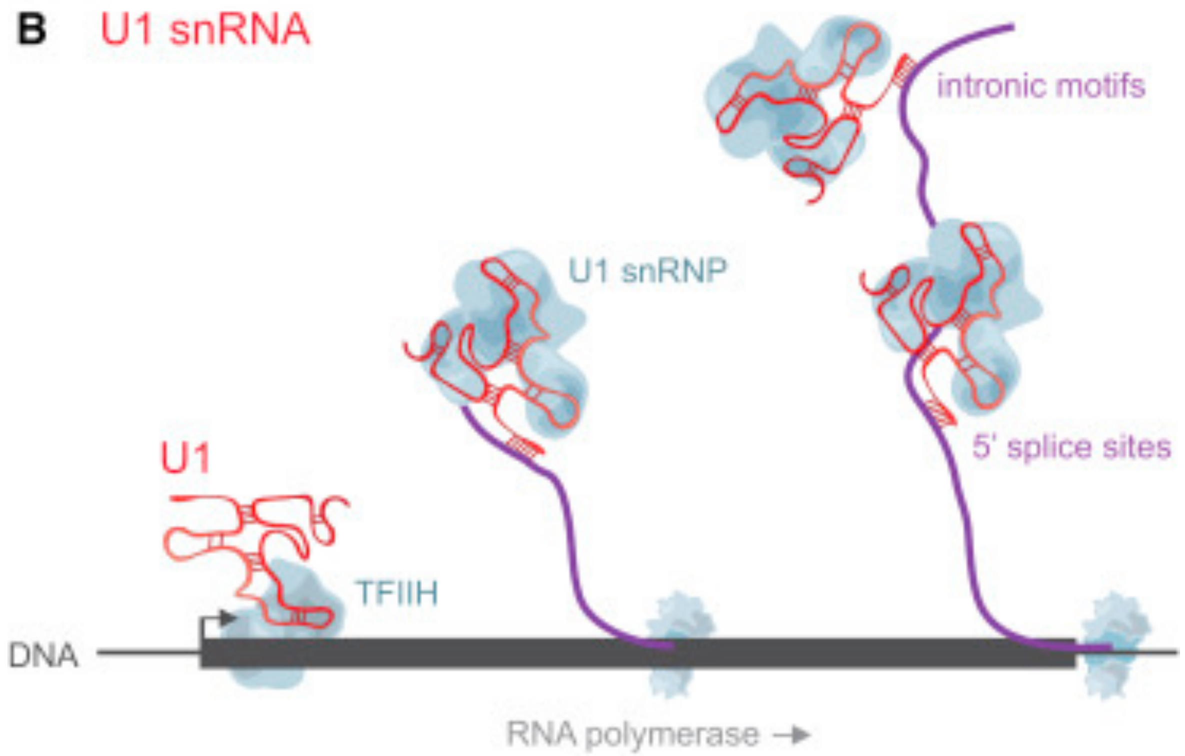




HOTAIR non coding RNA blocks interaction between Androgen receptor and MDM2 preventing its degradation

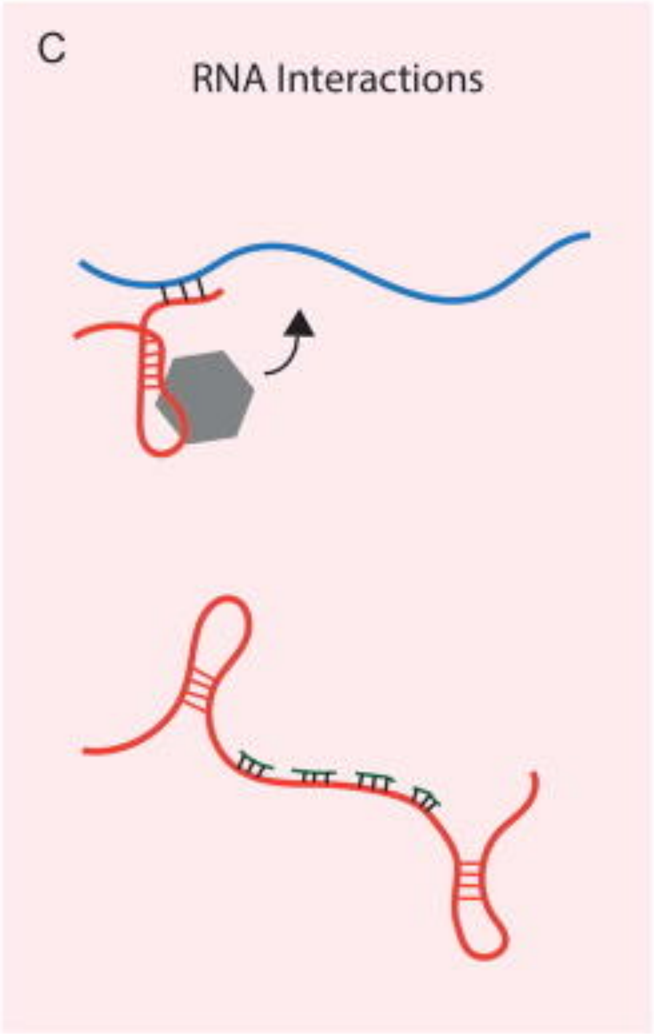
ncRNAs
Can regulate effector genes using different pathways

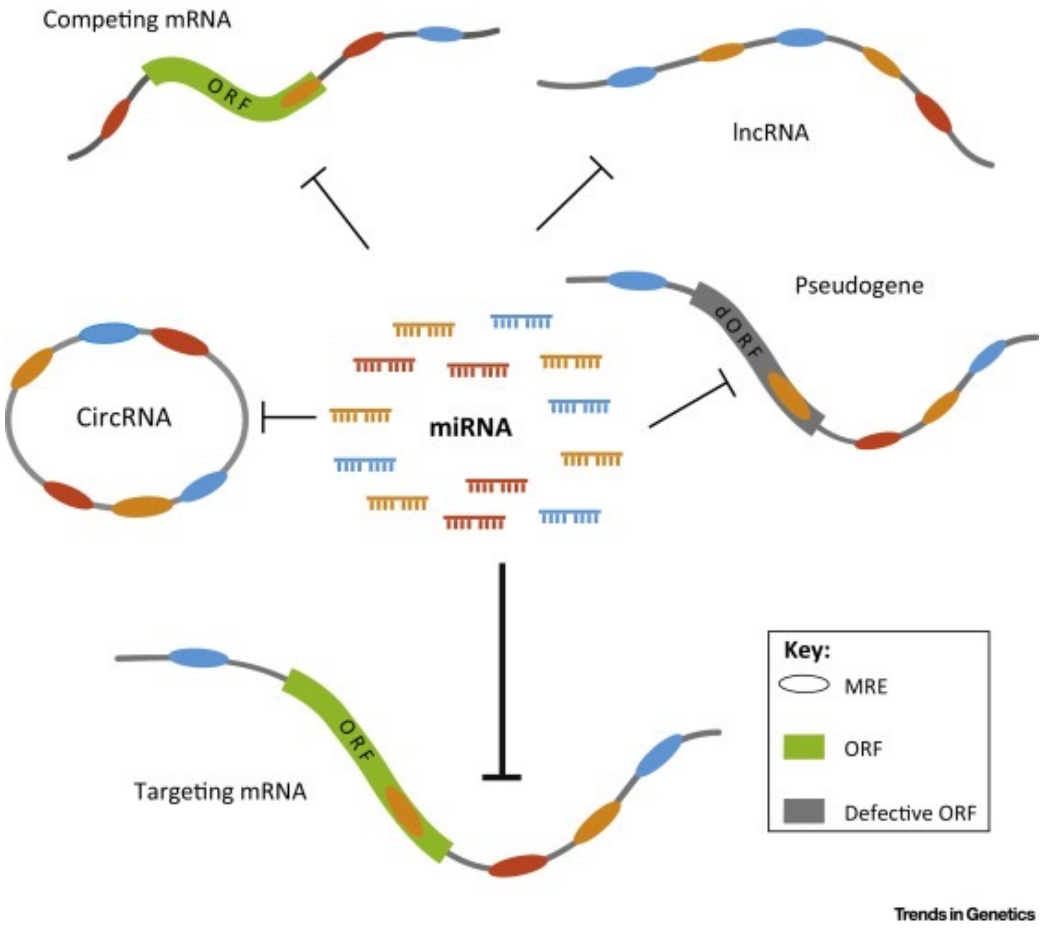




U1 ncRNA interacts with nascent RNA to control splicing

ncRNAs. Can regulate effector genes using different pathways





Competing RNA targeting as a form of regulation

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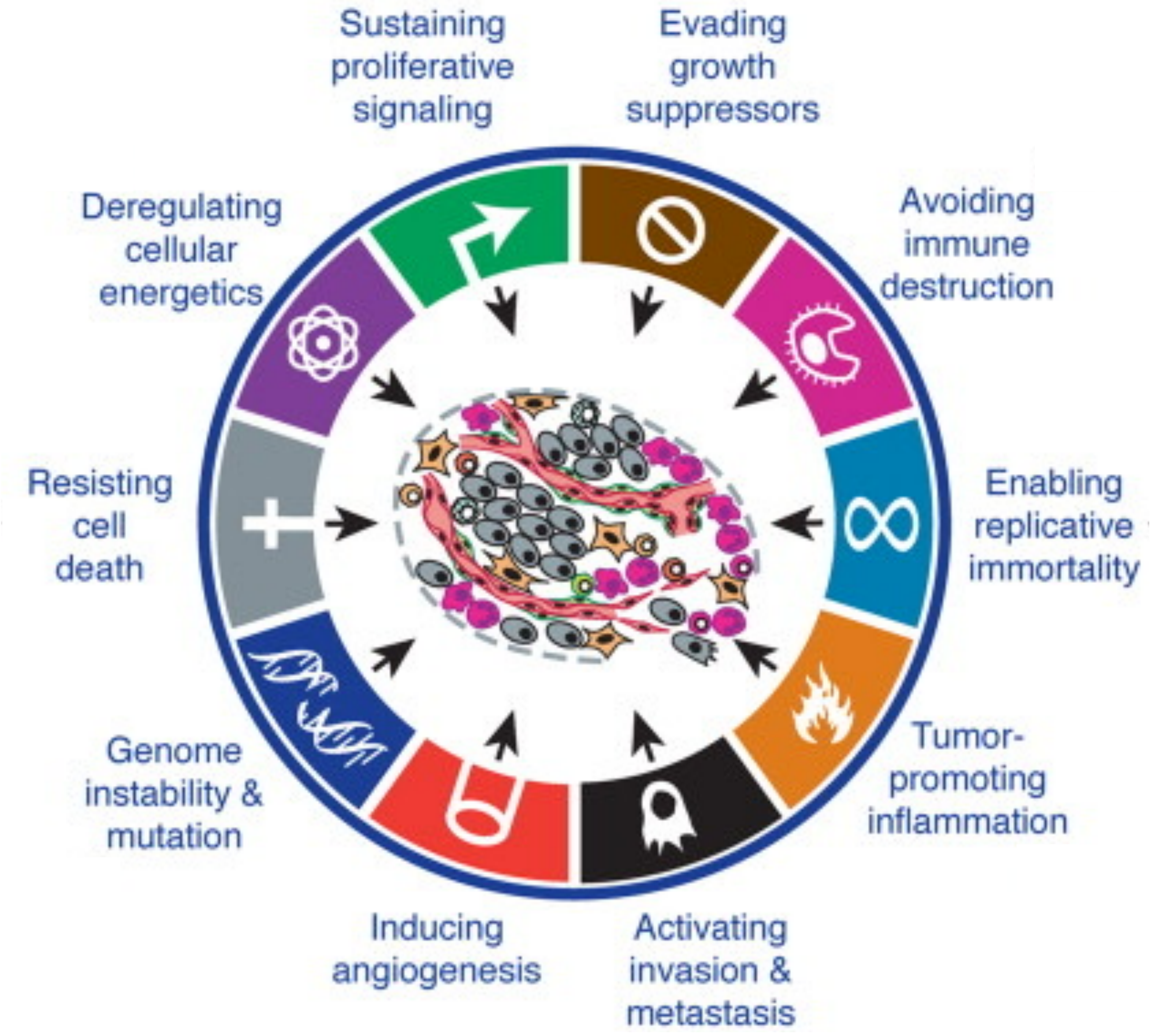
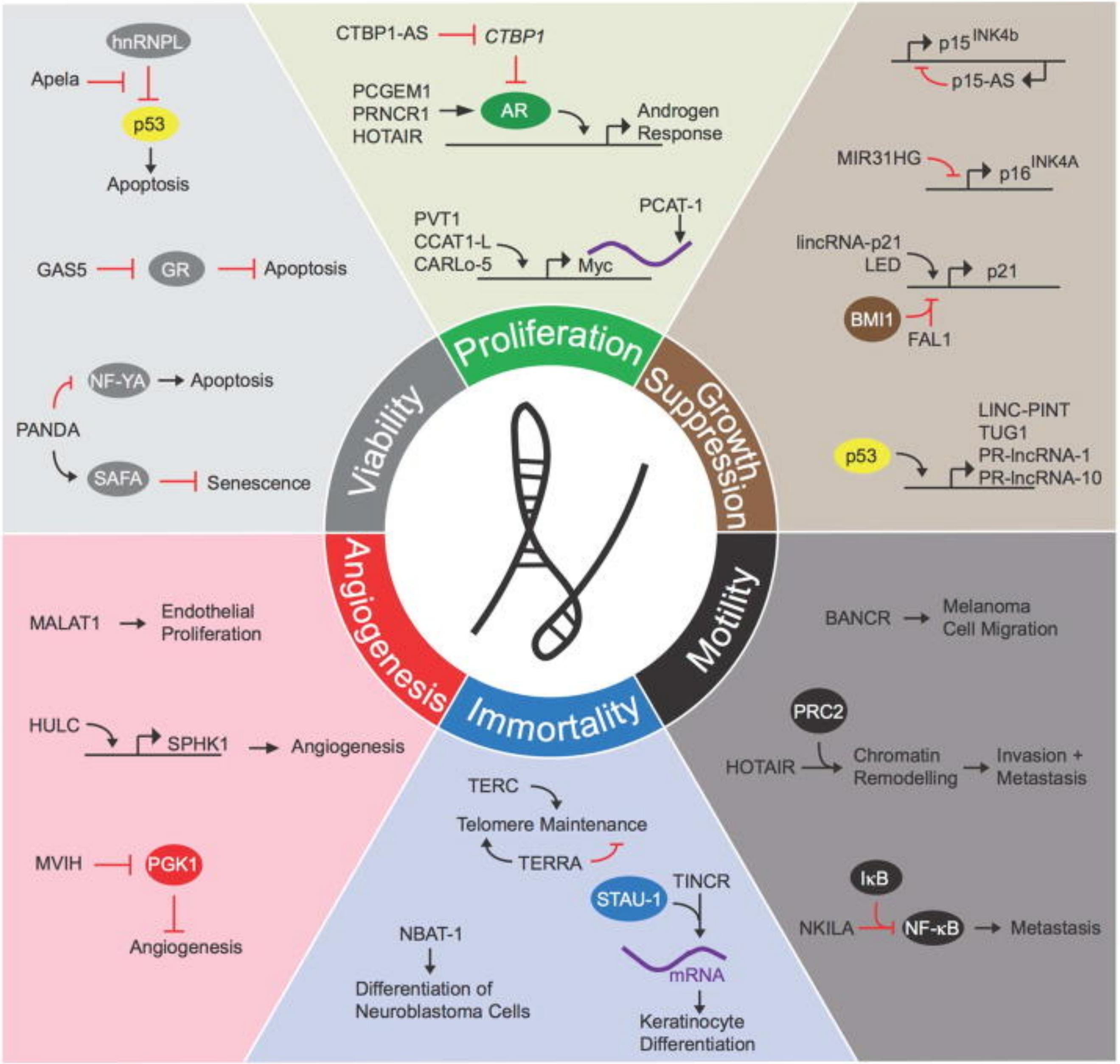
ncRNAs

Can regulate effector genes using different pathways



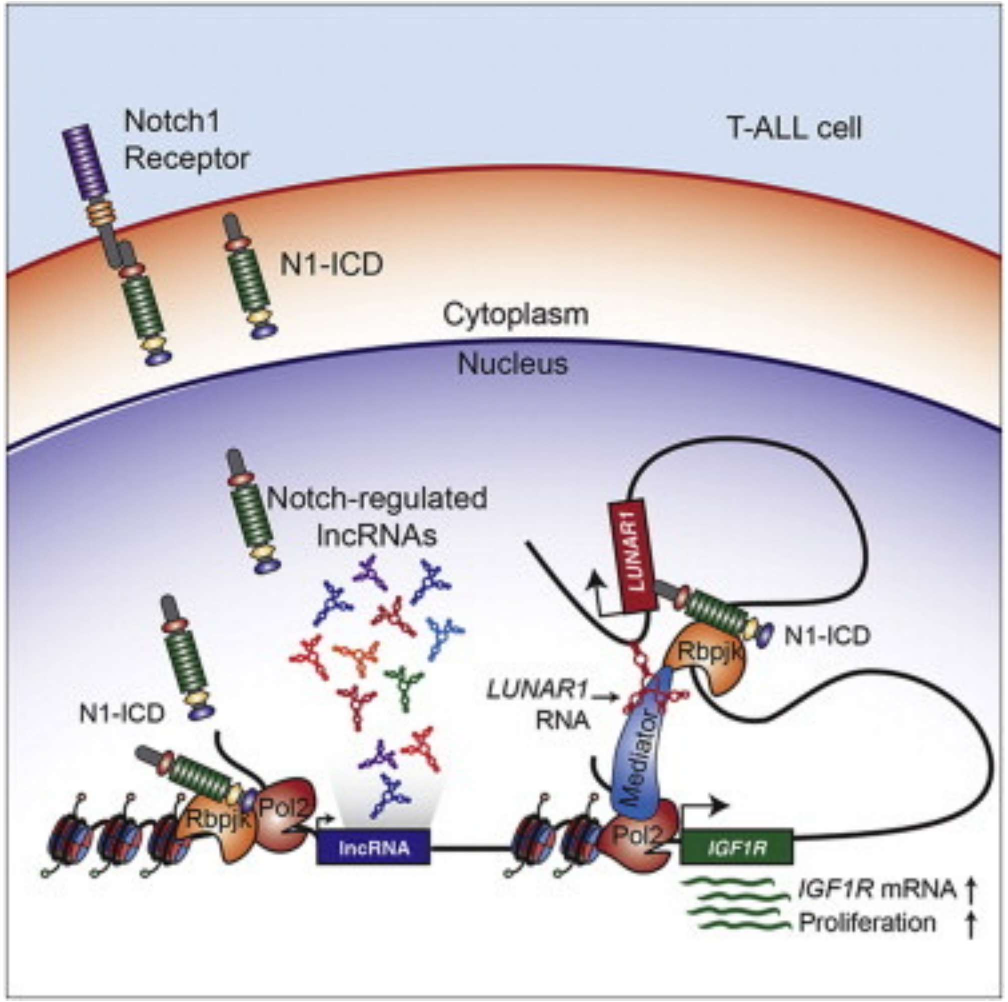
ncRNAs regulates cancer altering any of the of the pathways than promote cancer



hnRNPL
Apela
p53
Apoptosis
GAS5
GR
Apoptosis
NF-YA
Apoptosis
PANDA
SAFA
Senescence
CTBP1-AS
CTBP1
PCGEM1
PRNCR1
HOTAIR
AR
Androgen Response
PVT1
CCAT1-L
CARLo-5
PCAT-1
Myc
p15INK4b
p15-AS
MIR31HG
p16INK4A
lincRNA-p21
LED
p21
BMI1
FAL1
LINC-PINT
TUG1
PR-lncRNA-1
PR-lncRNA-10
p53
Proliferation
Growth Suppression
Viability
Angiogenesis
Immortality
Motility
MALAT1
Endothelial Proliferation
HULC
SPHK1
Angiogenesis
MVIH
PGK1
Angiogenesis
TERC
Telomere Maintenance
TERRA
STAU-1
TINCR
mRNA
Keratinocyte Differentiation
NBAT-1
Differentiation of Neuroblastoma Cells
BANCR
Melanoma Cell Migration
PRC2
HOTAIR
Chromatin Remodelling
Invasion + Metastasis
IκB
NKILA
NF-κB
Metastasis

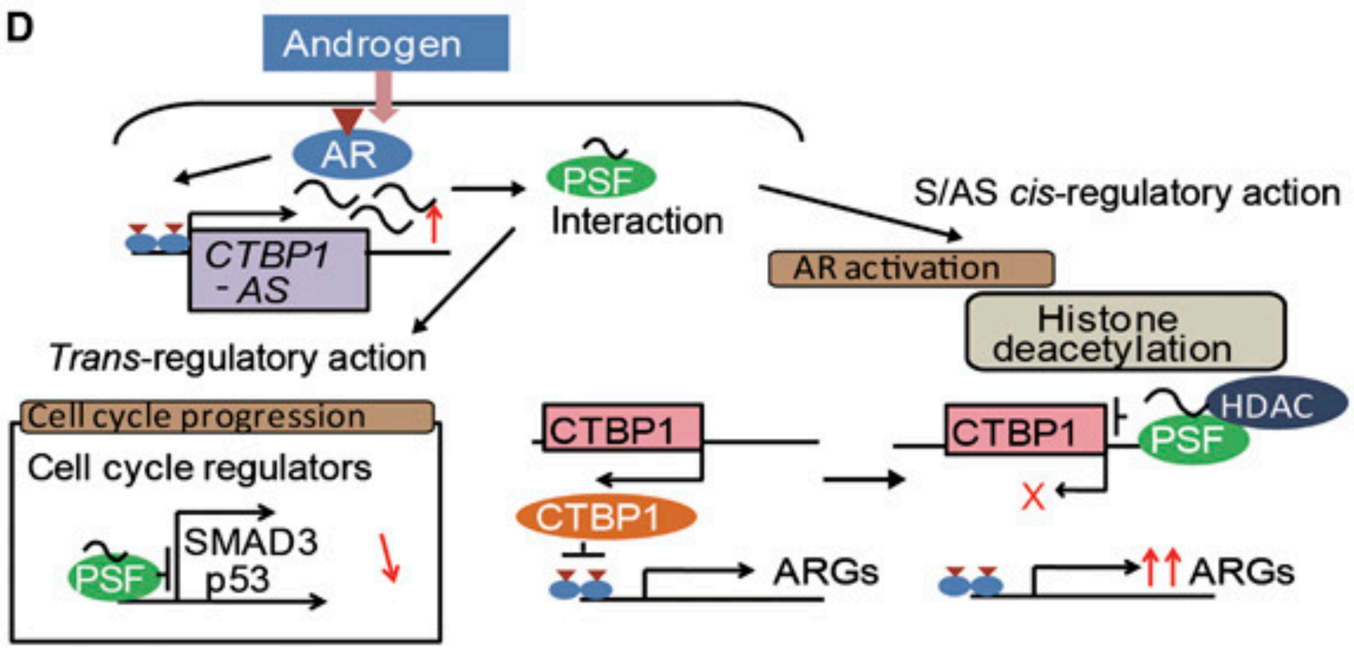
Proliferation circuits and ncRNAs.

Proliferation circuits and ncRNAs.



In T cell acute lymphoblastic leukemia (T-ALL) a specific Notch-regulated lncRNA, LUNAR1, is required for efficient T-ALL growth in vitro and in vivo due to its ability to enhance IGF1R mRNA expression and sustain IGF1 signaling.

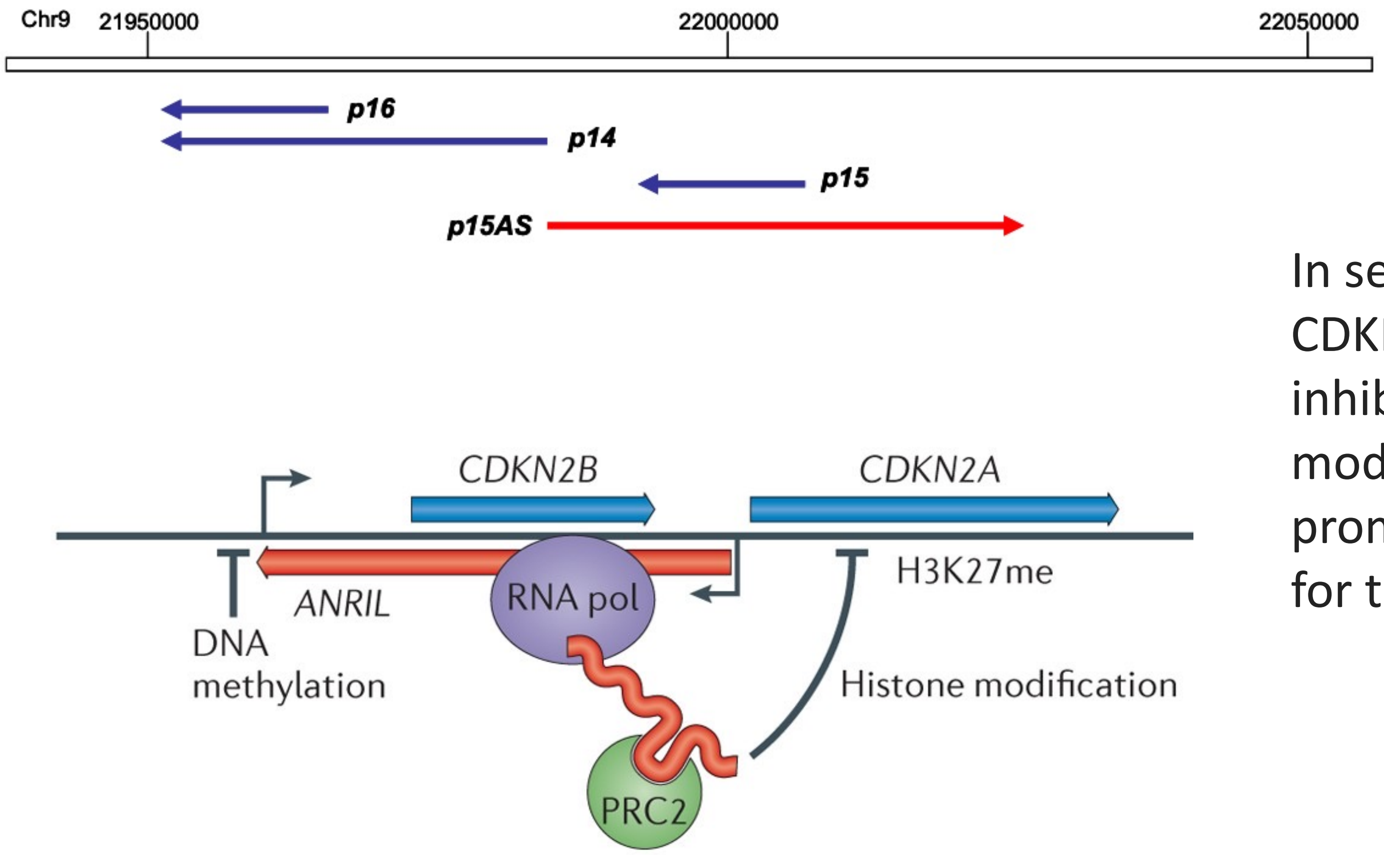
Proliferation circuits and ncRNAs.



An androgen-responsive long ncRNA, CTBP1-AS, located in the AS region of C-terminal binding protein 1 (CTBP1), which is a corepressor for androgen receptor. CTBP1-AS is predominantly localized in the nucleus and its expression is generally upregulated in prostate cancer. CTBP1-AS promotes both hormone-dependent and castration-resistant tumour growth. Mechanistically, CTBP1-AS directly represses CTBP1 expression by recruiting the RNA-binding transcriptional repressor PSF together with histone deacetylases. CTBP1-AS also exhibits global androgen-dependent functions by inhibiting tumour-suppressor genes via the PSF-dependent mechanism thus promoting cell cycle progression

Evading growth suppression circuits

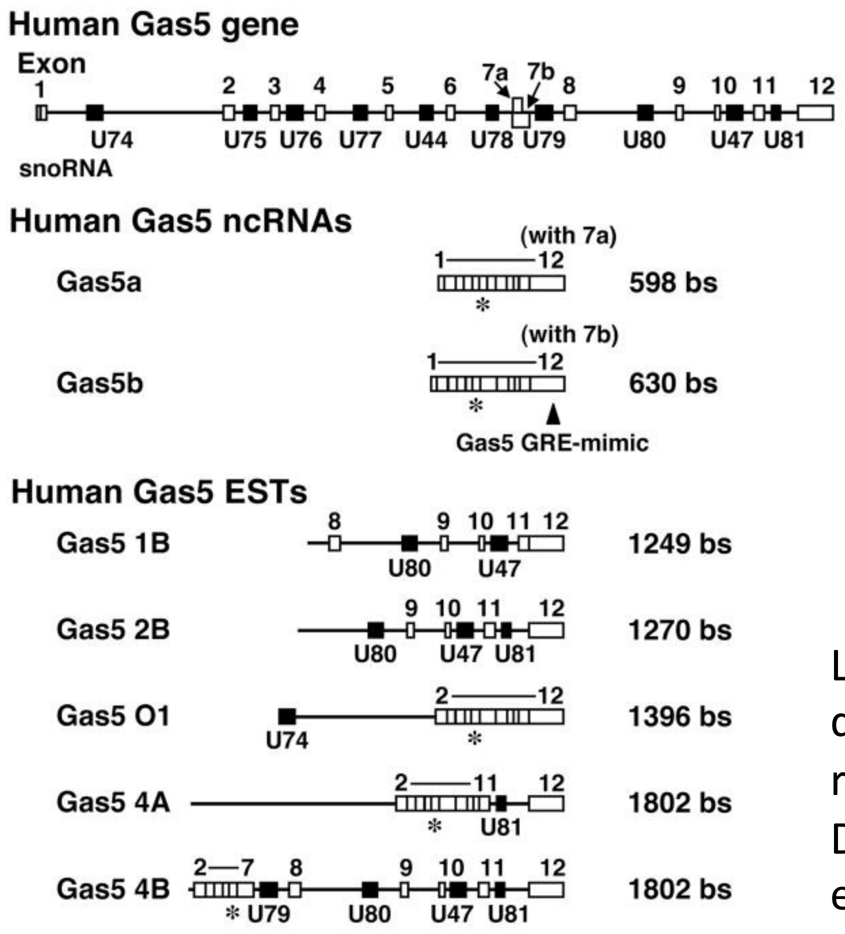
Evading growth suppressors

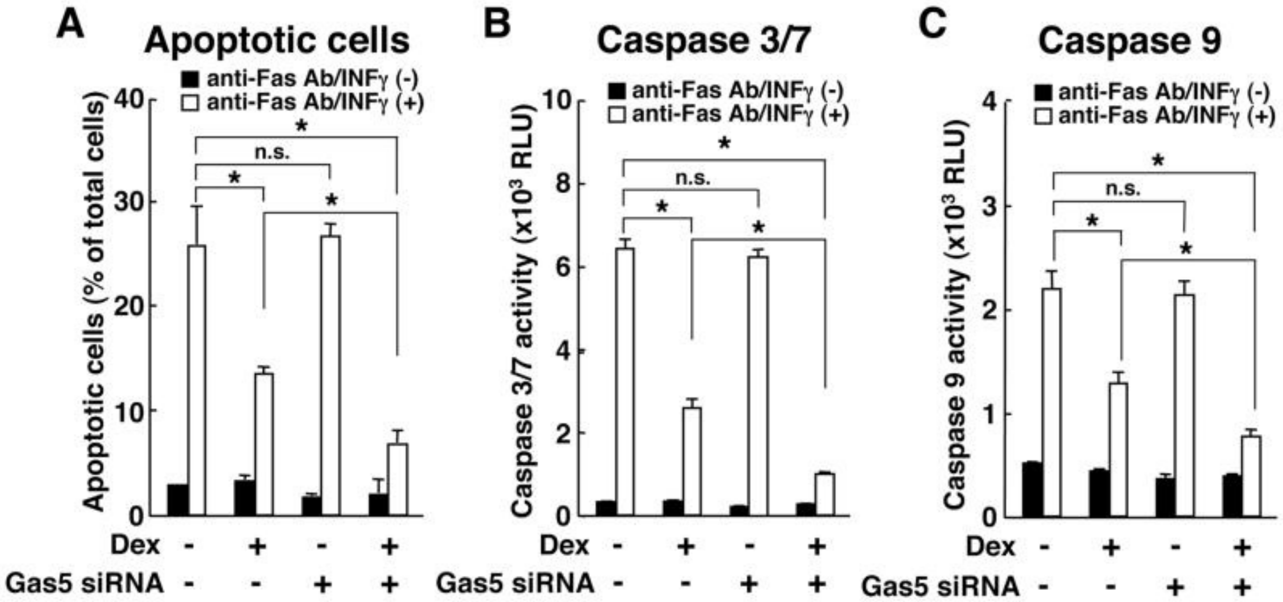


In several cancers, p15AS (ANRIL) inhibits CDKN2A/B, cycline dependent kinase inhibitor 2A/B, promoting histone modifications/DNA methylation in their promoters and thus removing the brakes for the cell cycle progression.

Resisting cell death

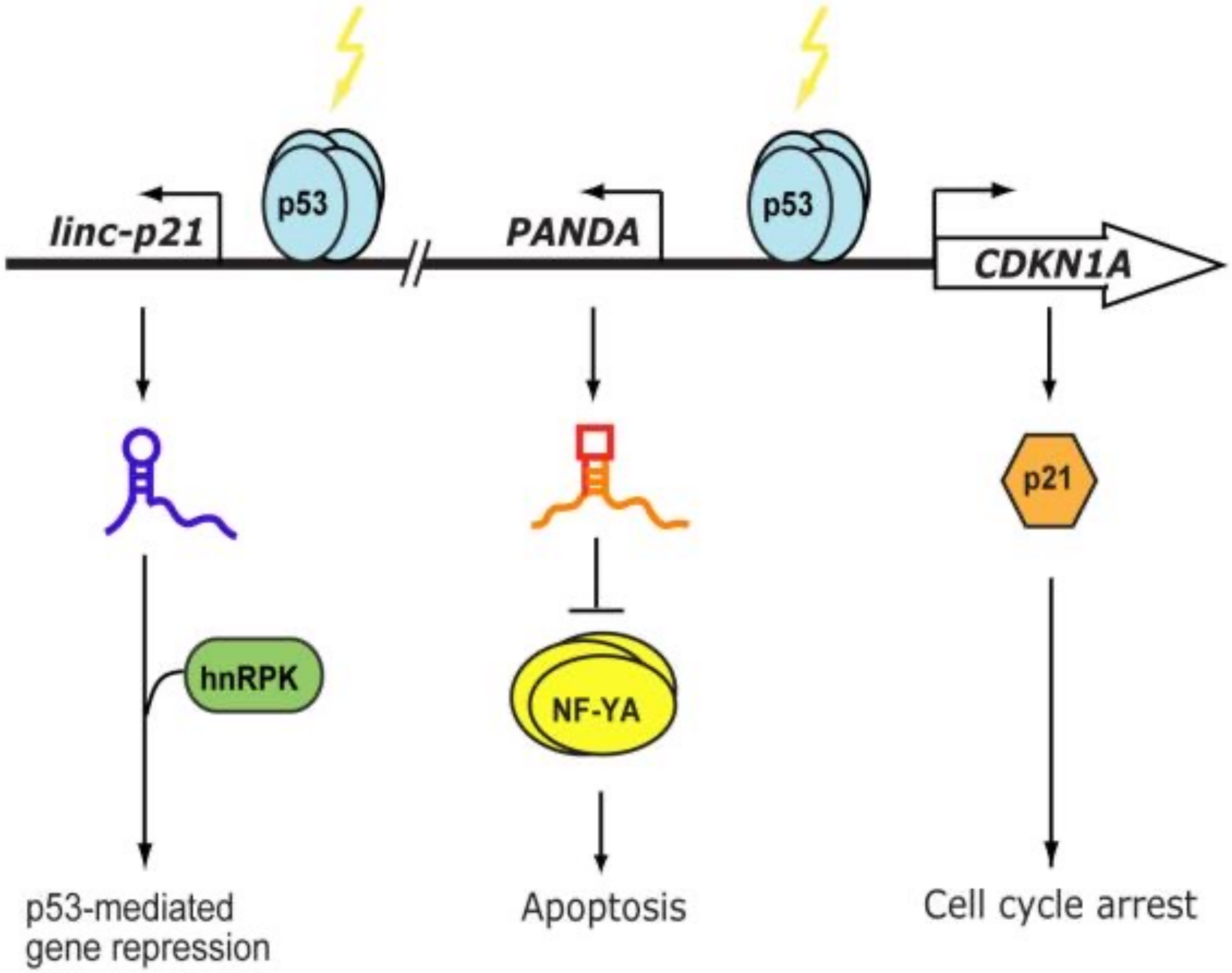
Resisting cell death





LncRNA Growth arrest specific 5 (Gas5) is induced in cells arrested by nutrient deprivation or withdrawal of growth factors. Gas5 blocks glucocorticoid responsive gene expression by binding to the glucocorticoid receptor's (GR's) DNA binding domain and acting as a decoy This blockade of GR decreases expression of the cellular inhibitor of apoptosis 2 thereby enhancing apoptosis under stressed conditions in normal cells. However, suppression of Gas5 in human breast cancer cells relative to adjacent normal breast tissue may support the enhanced viability of breast cancer cells in the nutrient poor tumor microenvironment

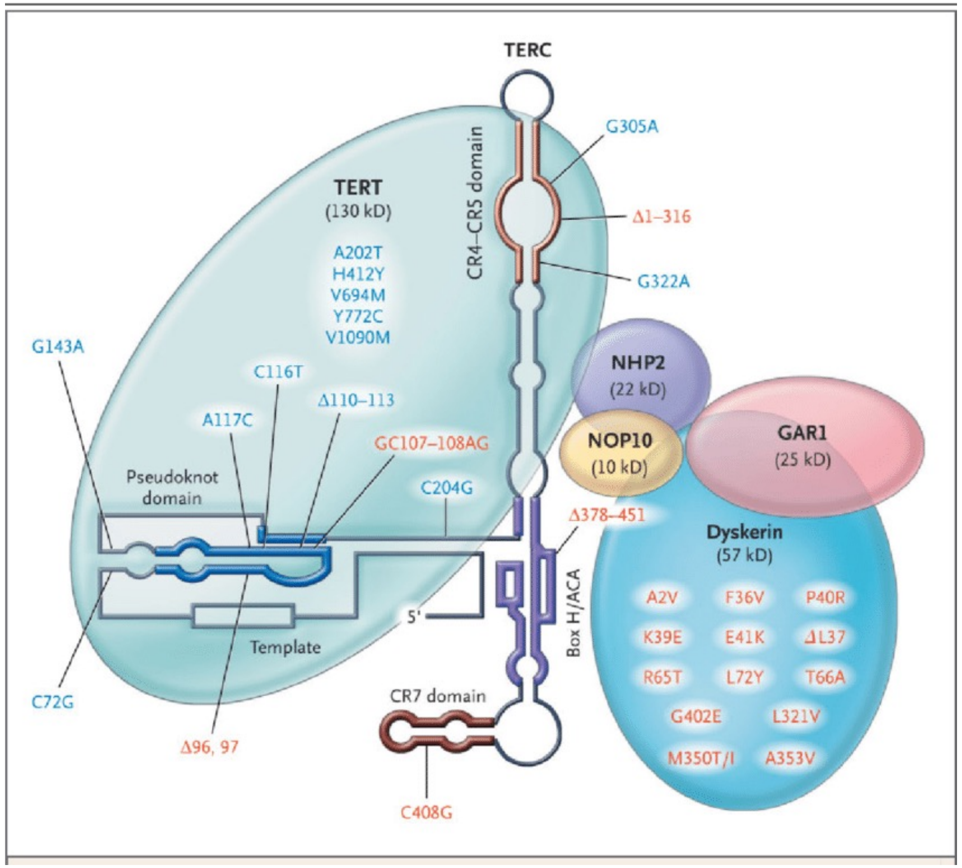
Resisting cell death

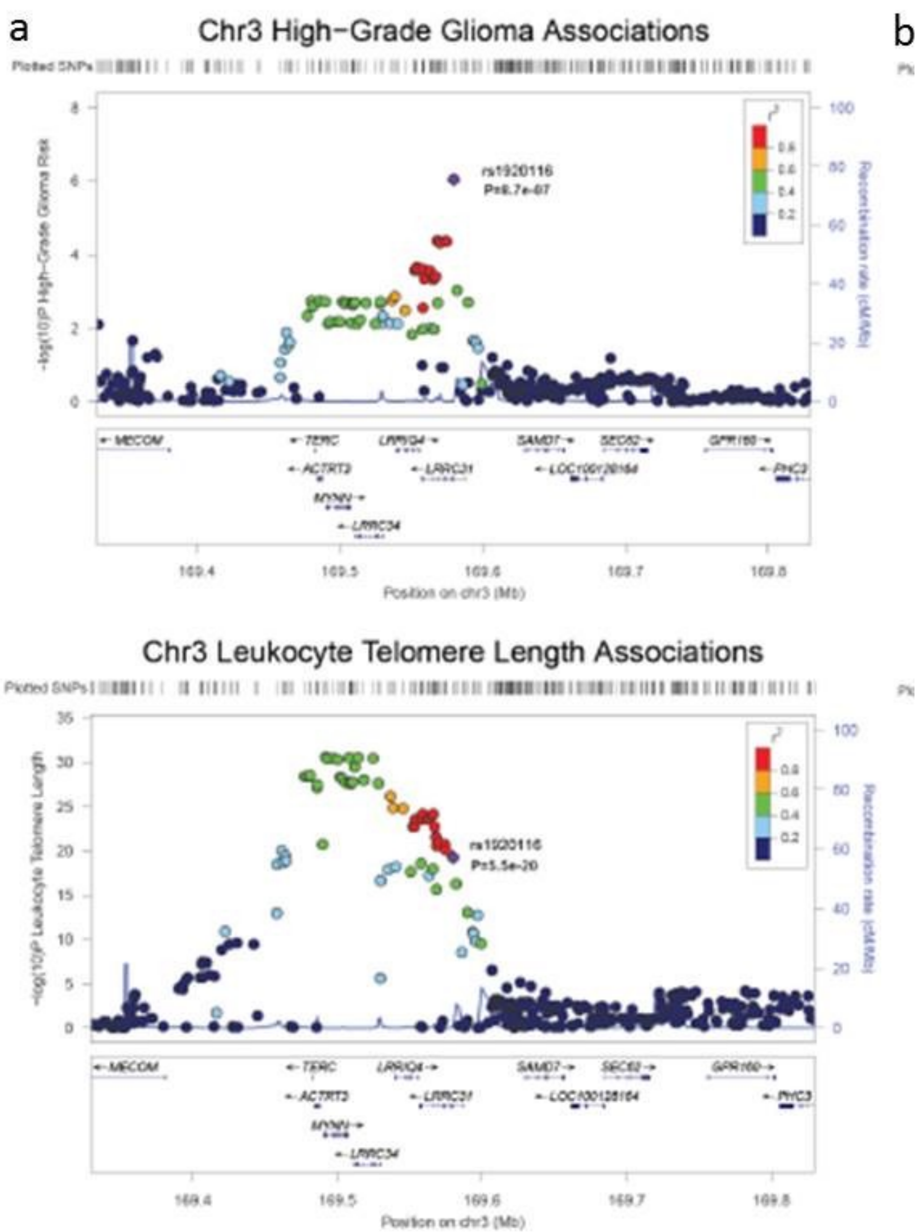


After DNA damage, p53 binding at the *CDKN1A* locus coordinately activates transcription of *CDKN1A* as well as noncoding transcripts PANDA and linc-p21. CDKN1A mediates cell cycle arrest, PANDA blocks apoptosis through NF-YA, and linc-p21 mediates gene silencing through recruitment of hnRPK.

Immortality circuits

Inmortality circuits

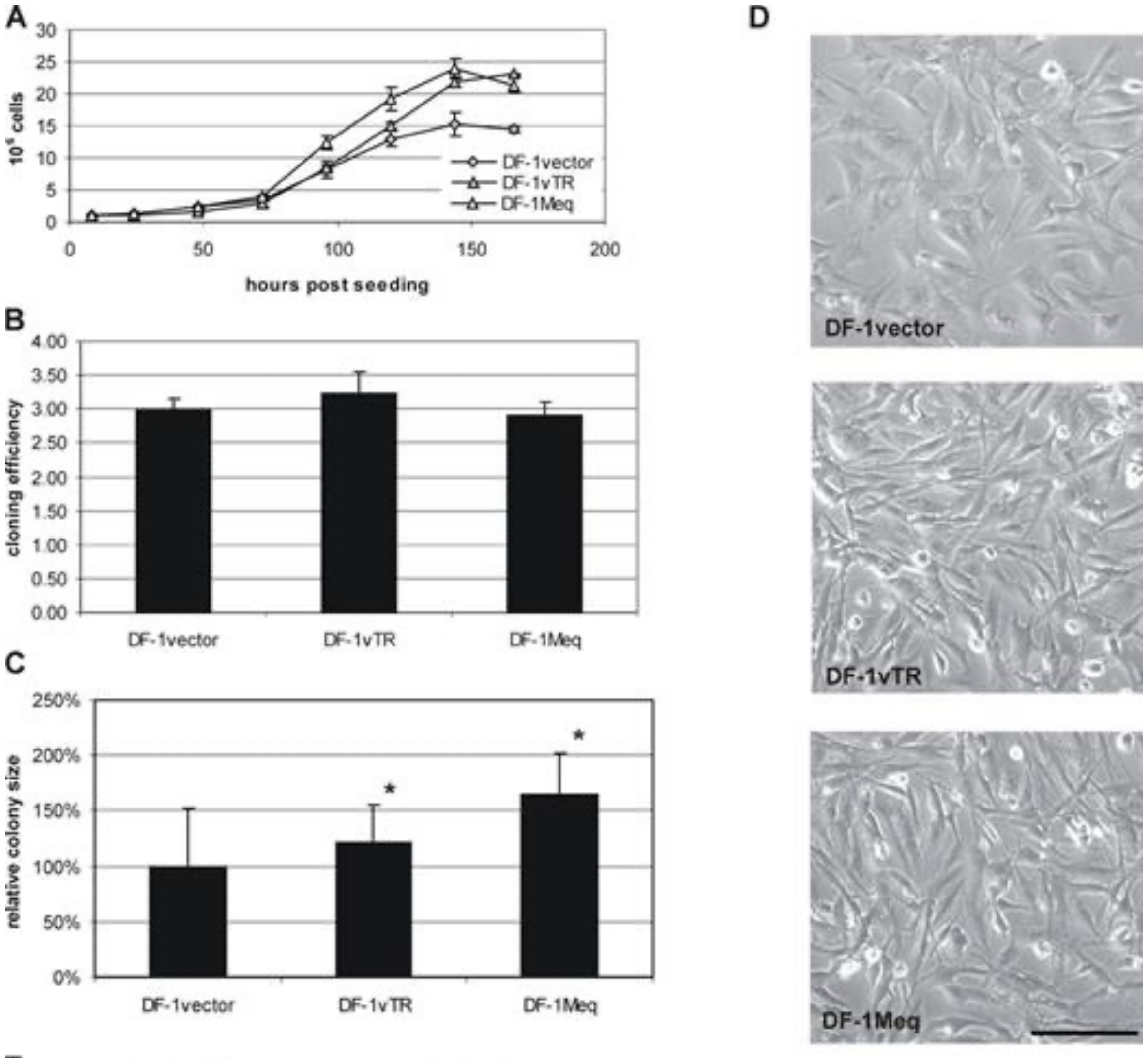




Point mutations in the TERC RNA (who forms part of the telomerase) is related with glioma formation.

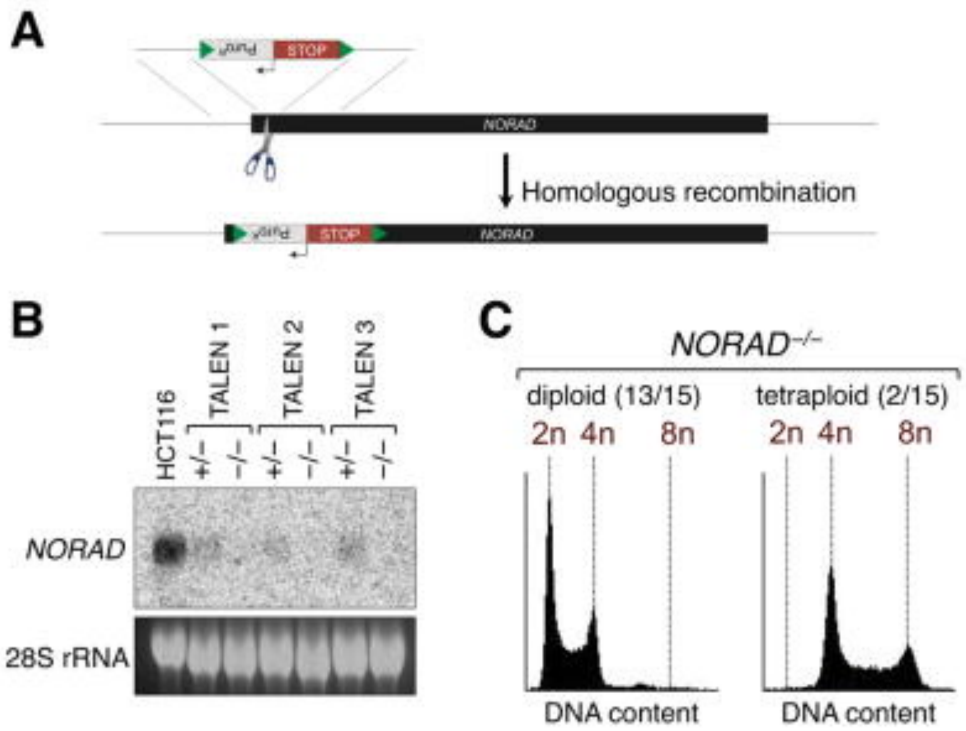
Inmortality circuits

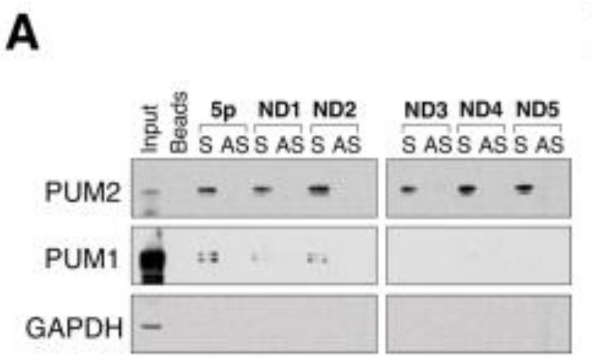
In fact it is enough with the infection of some viral RNA telomerase to promote malignant T cell lymphomagenesis

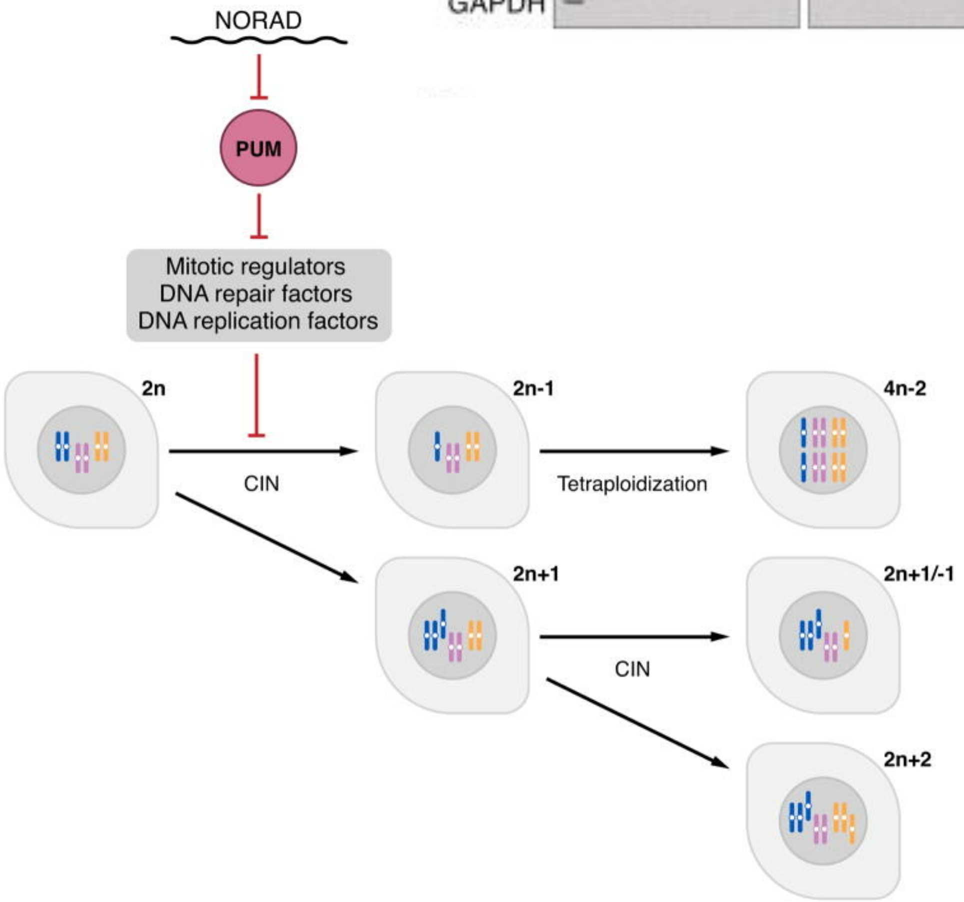


Genome stability and supression

Immortality circuits.







NORAD is a ncRNA that inhibits PUMILIO protein that is a protein than repress post transcriptionally DNA Repair/replication factors.

Motility (invasion and metastasis)

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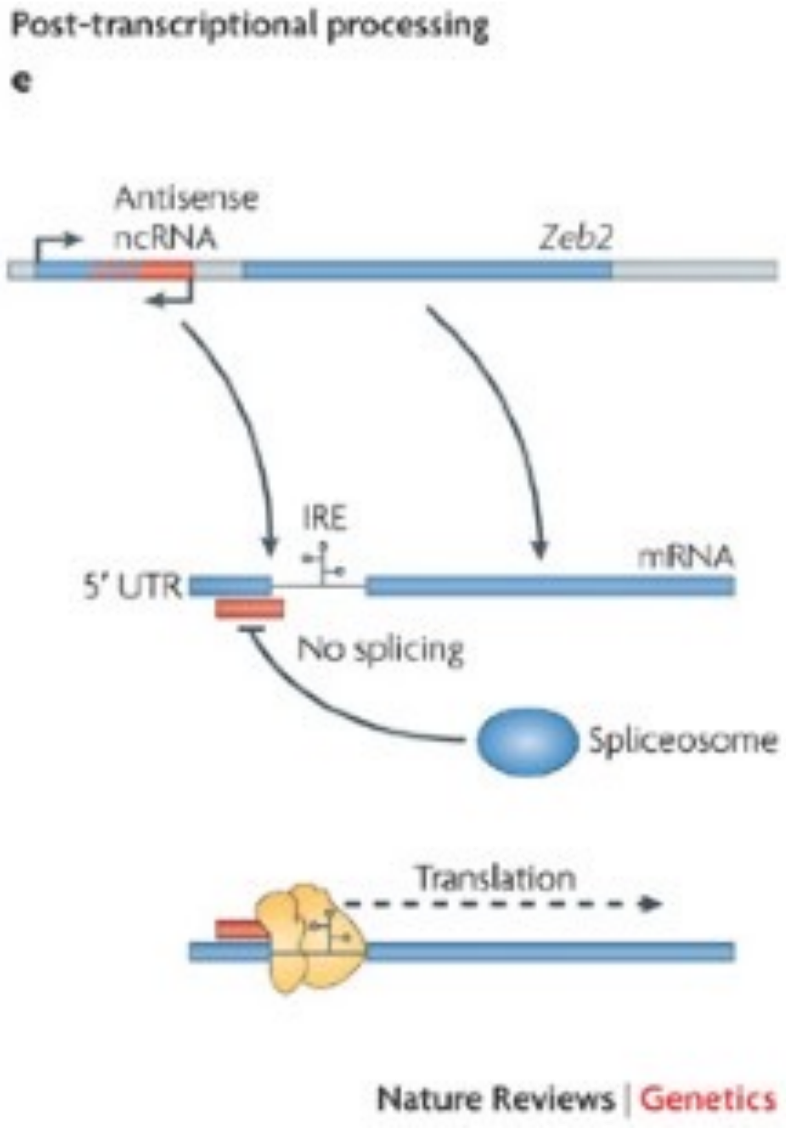

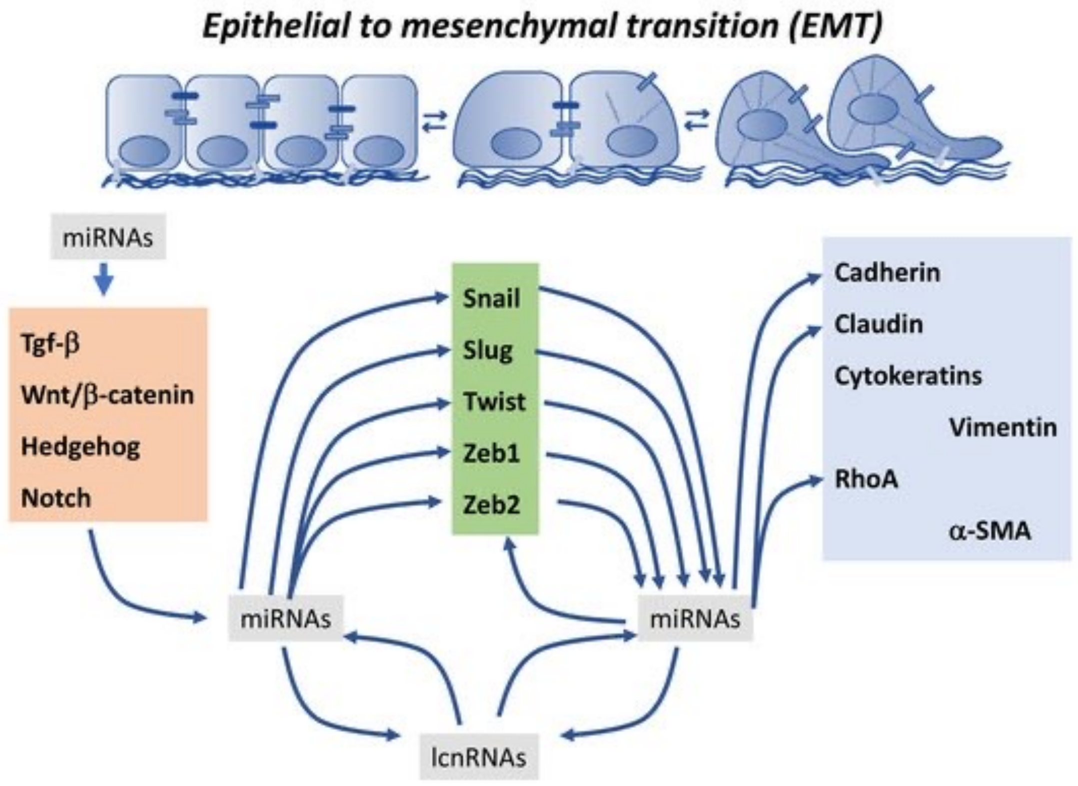

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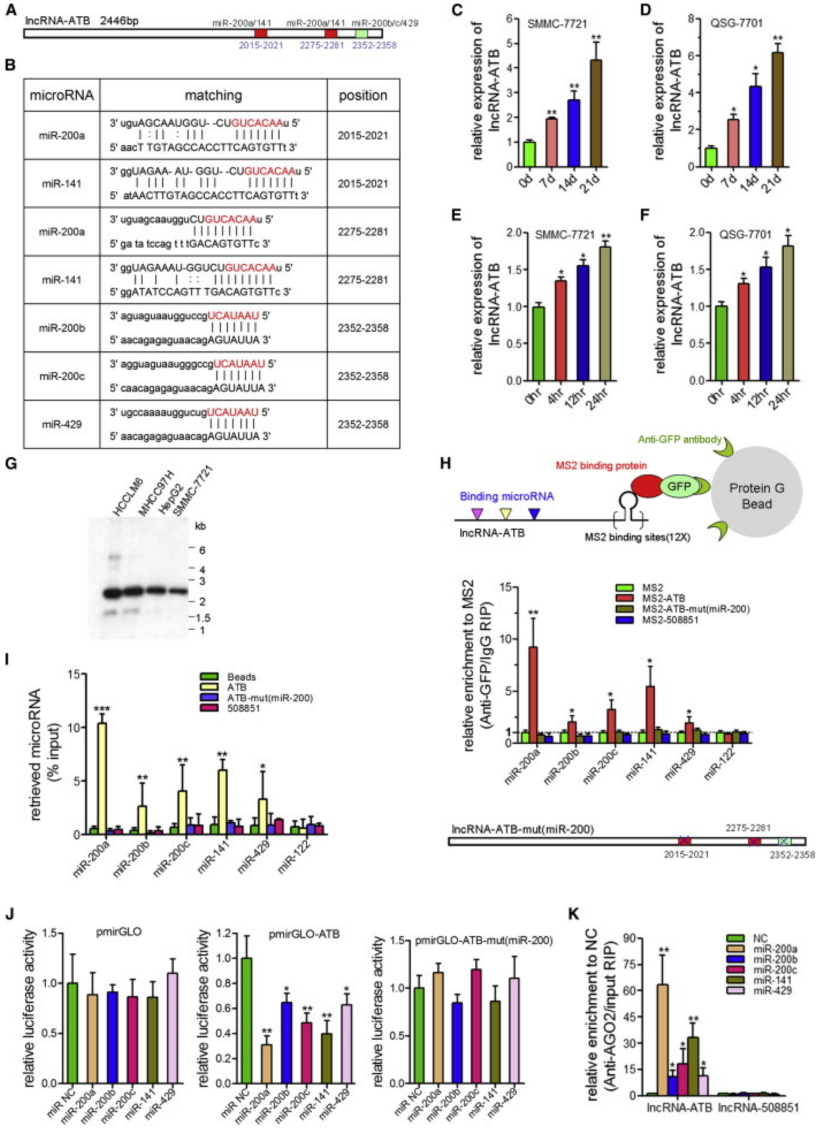
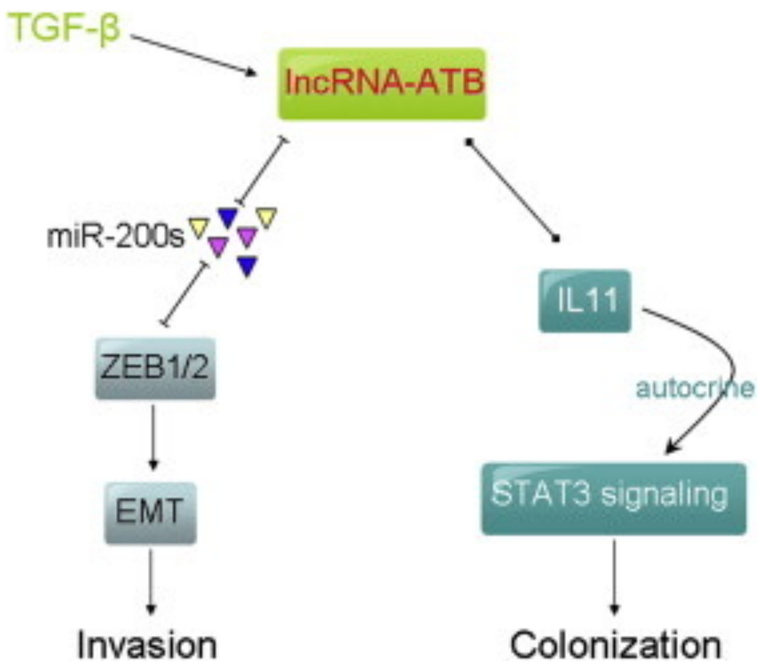
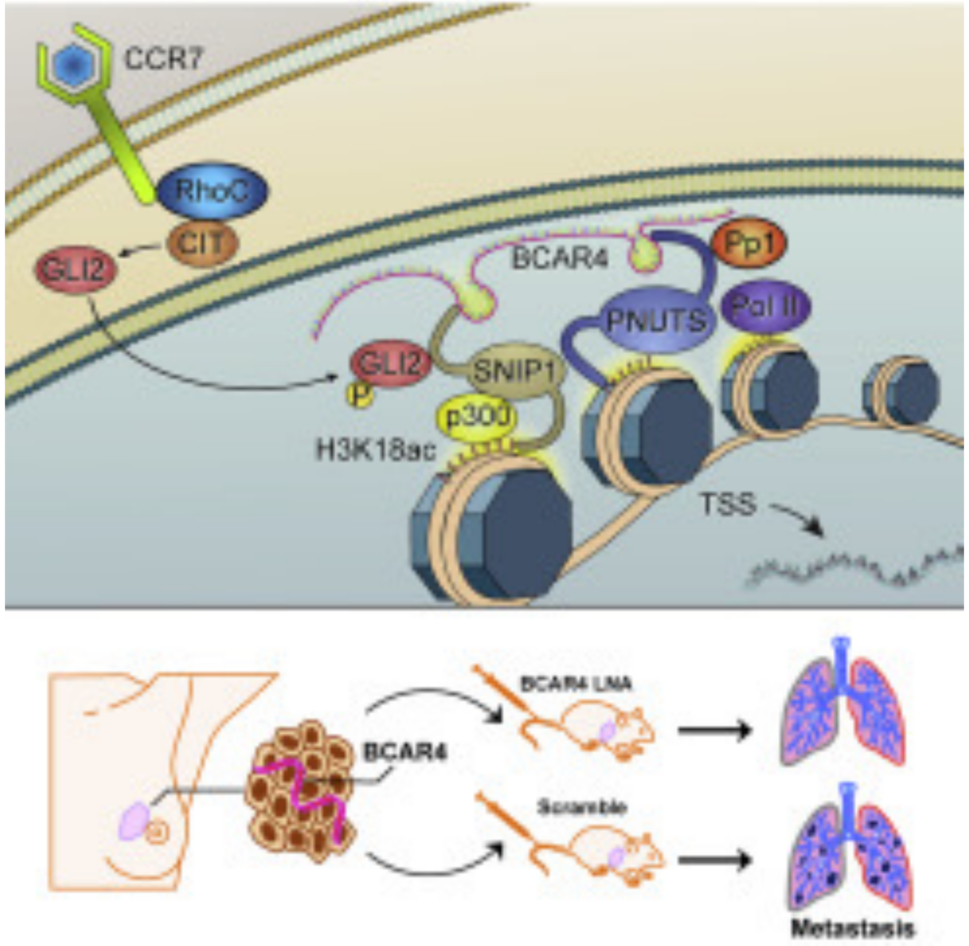


The lncRNA-activated by TGF-β (lncRNA-ATB) was upregulated in hepatocellular carcinoma (HCC) metastases and associated with poor prognosis. lncRNA-ATB upregulated ZEB1 and ZEB2 by competitively binding the miR-200 family and then induced EMT and invasion. In addition, lncRNA-ATB promoted organ colonization of disseminated tumor cells by binding IL-11 mRNA, autocrine induction of IL-11, and triggering STAT3 signaling. Globally, lncRNA-ATB promotes the invasion-metastasis cascade.



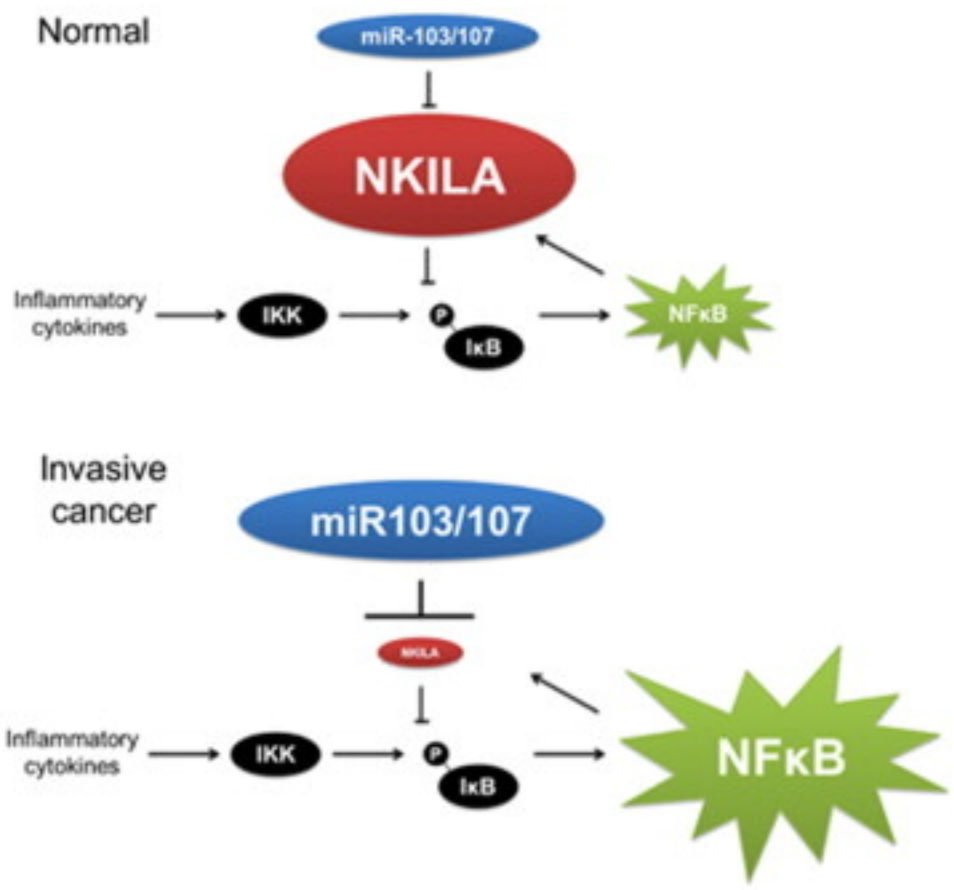
Evading immune response

Evading immune response



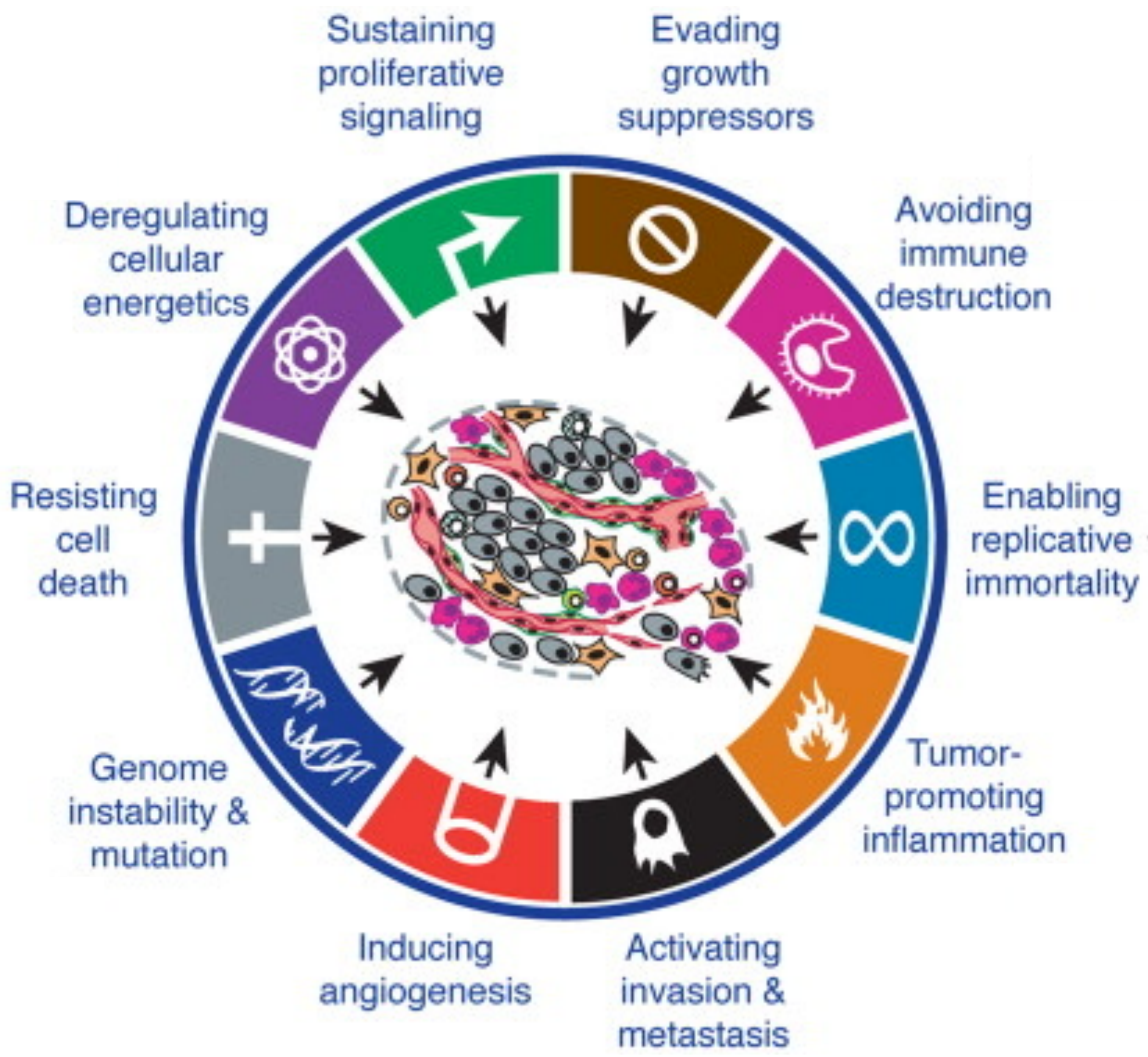
lncRNA BCAR4 in breast cancer metastasis that is mediated by chemokine-induced binding of BCAR4 to two transcription factors with extended regulatory consequences. BCAR4 binding of SNIP1 and PNUTS in response to CCL21 releases the SNIP1's inhibition of p300-dependent histone acetylation, which in turn enables the BCAR4-recruited PNUTS to bind H3K18ac and relieve inhibition of RNA Pol II via activation of the PP1 phosphatase. This mechanism activates a noncanonical Hedgehog/GLI2 transcriptional program that promotes cell migration. BCAR4 expression correlates with advanced breast cancers, and therapeutic delivery of locked nucleic acids(LNAs) targeting BCAR4 strongly suppresses breast cancer metastasis in mouse models.

Evading immune response

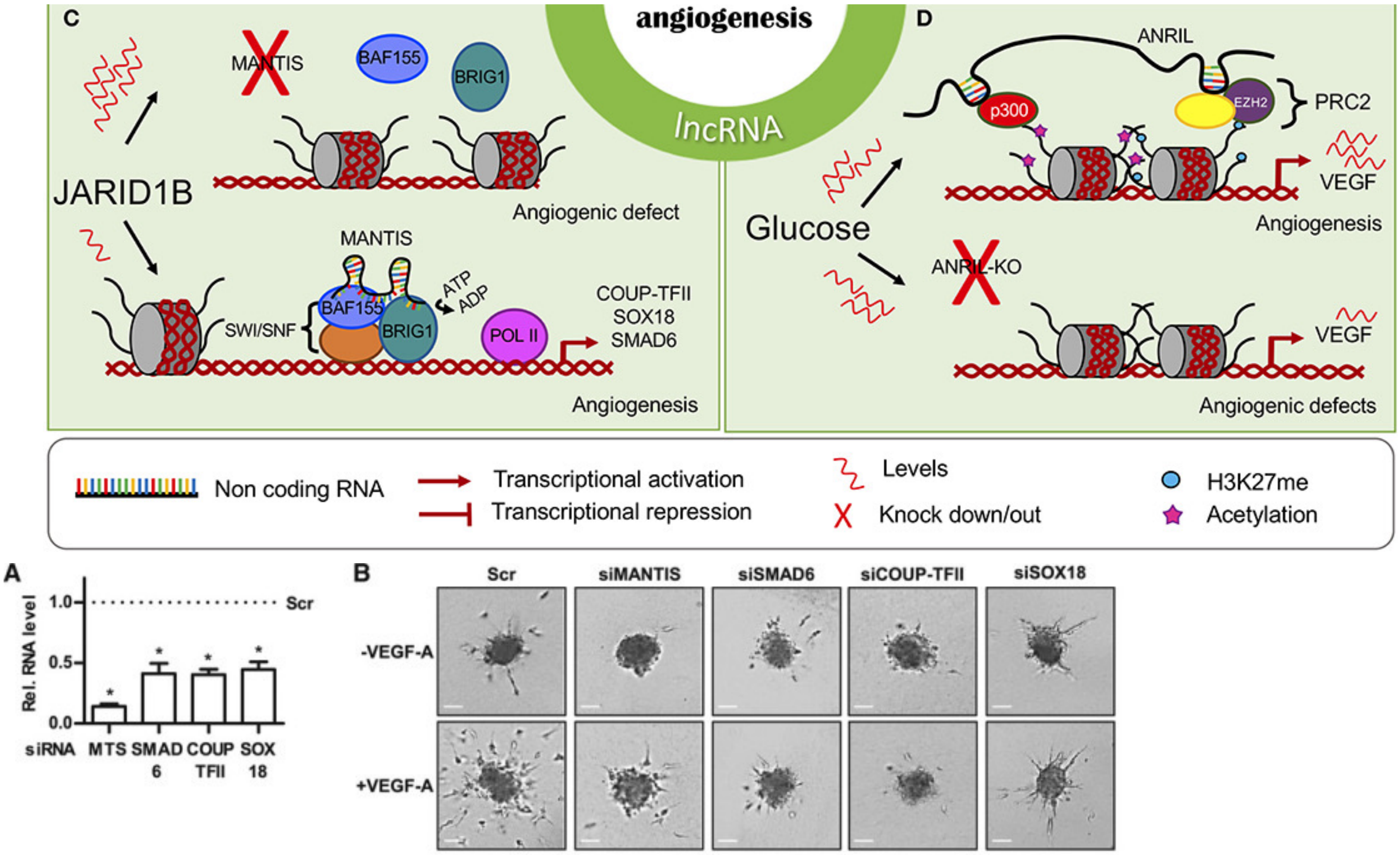


NKILA is an essential lncRNA that regulates NF-κB signaling and represses cancer-associated inflammation. NKILA binds to NF-κB/IκB complex and inhibits NF-κB signaling by masking the phosphorylation sites of IκB and stabilizing the complex. Nevertheless, NKILA expression is significantly decreased in many breast cancers, which is associated with cancer metastasis and poor patient prognosis. Our study has discovered a class of lncRNAs that directly regulate signaling pathways.

Inducing angiogenesis



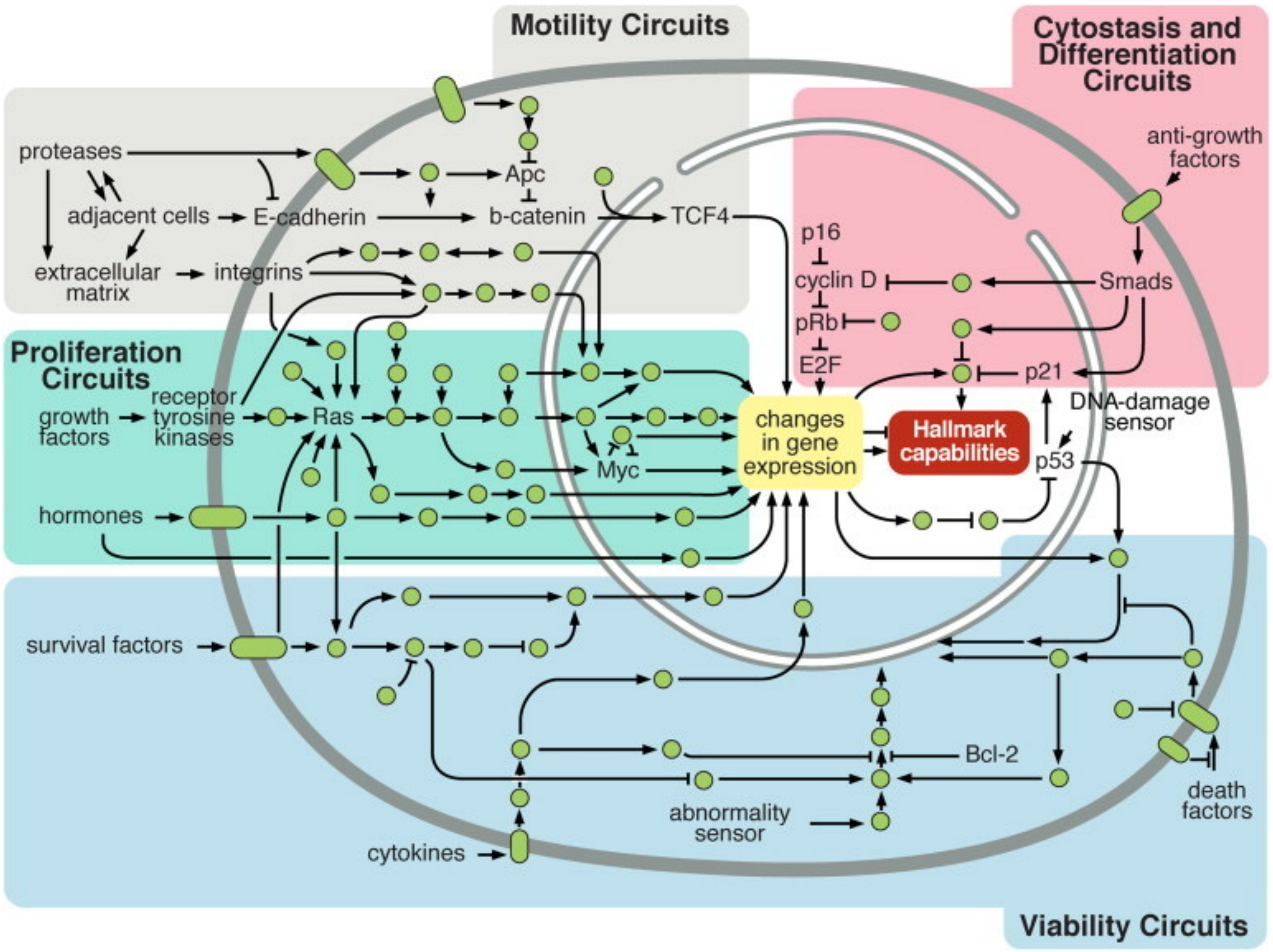
Inducing angiogenesis



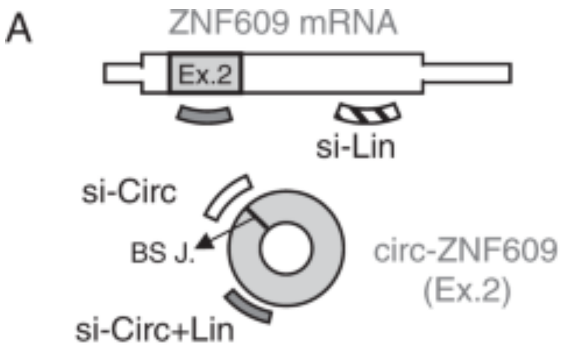
Hallmarks of cancer

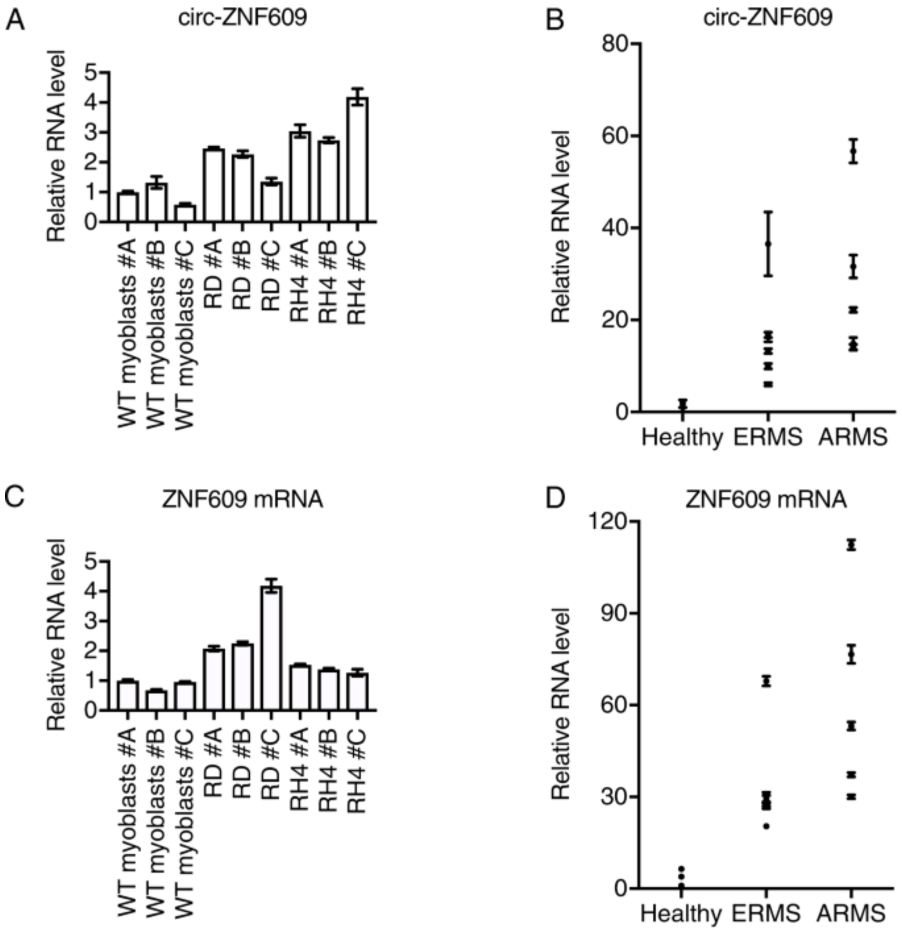
Hallmarks of cancer: are also valid for other types of ncRNAs

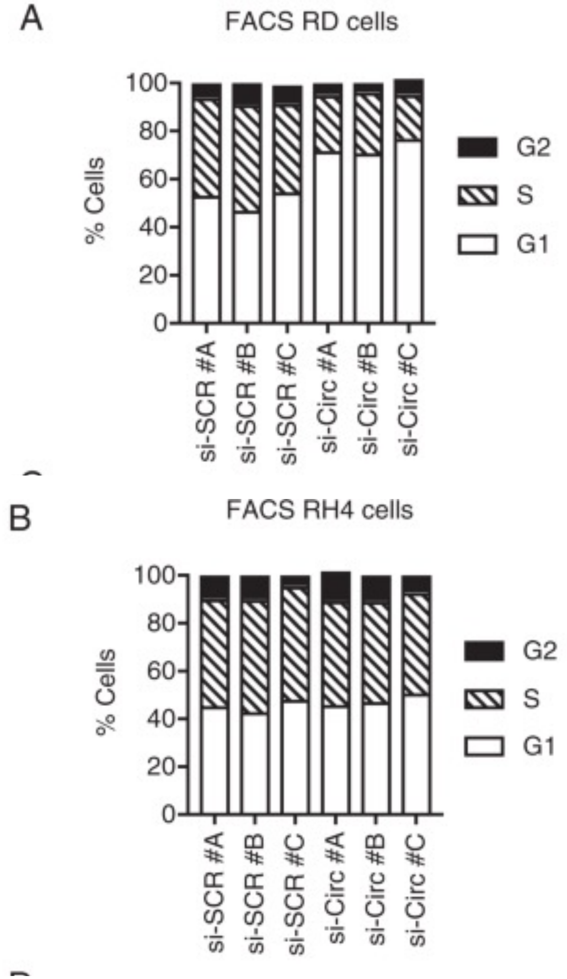
Cancer is fundamentally genetic disease than alter the cellular information flow to modify cellular homeostasis and promote cell growth



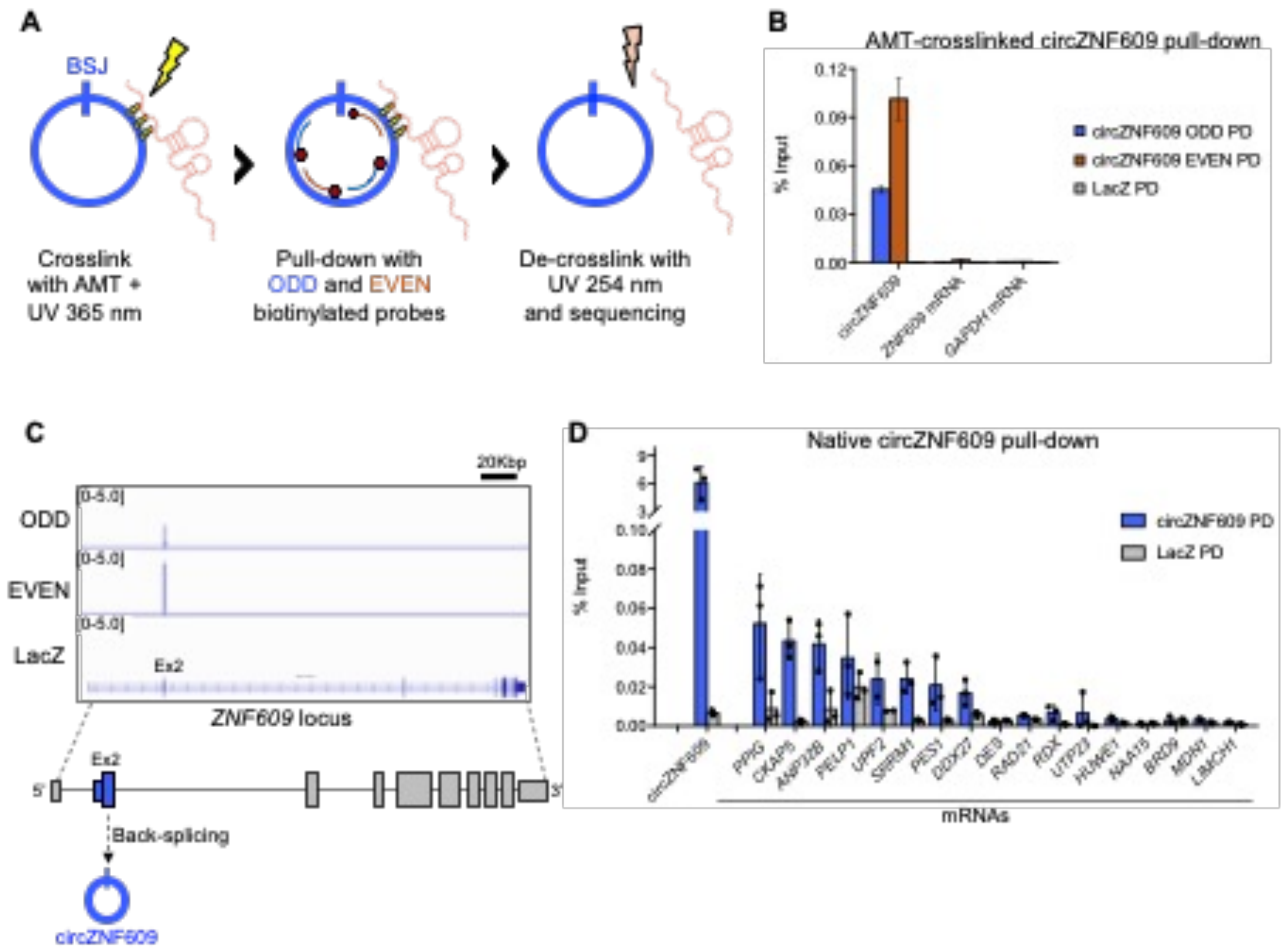
But things are not always so straight forward: circZNF609 in RMS





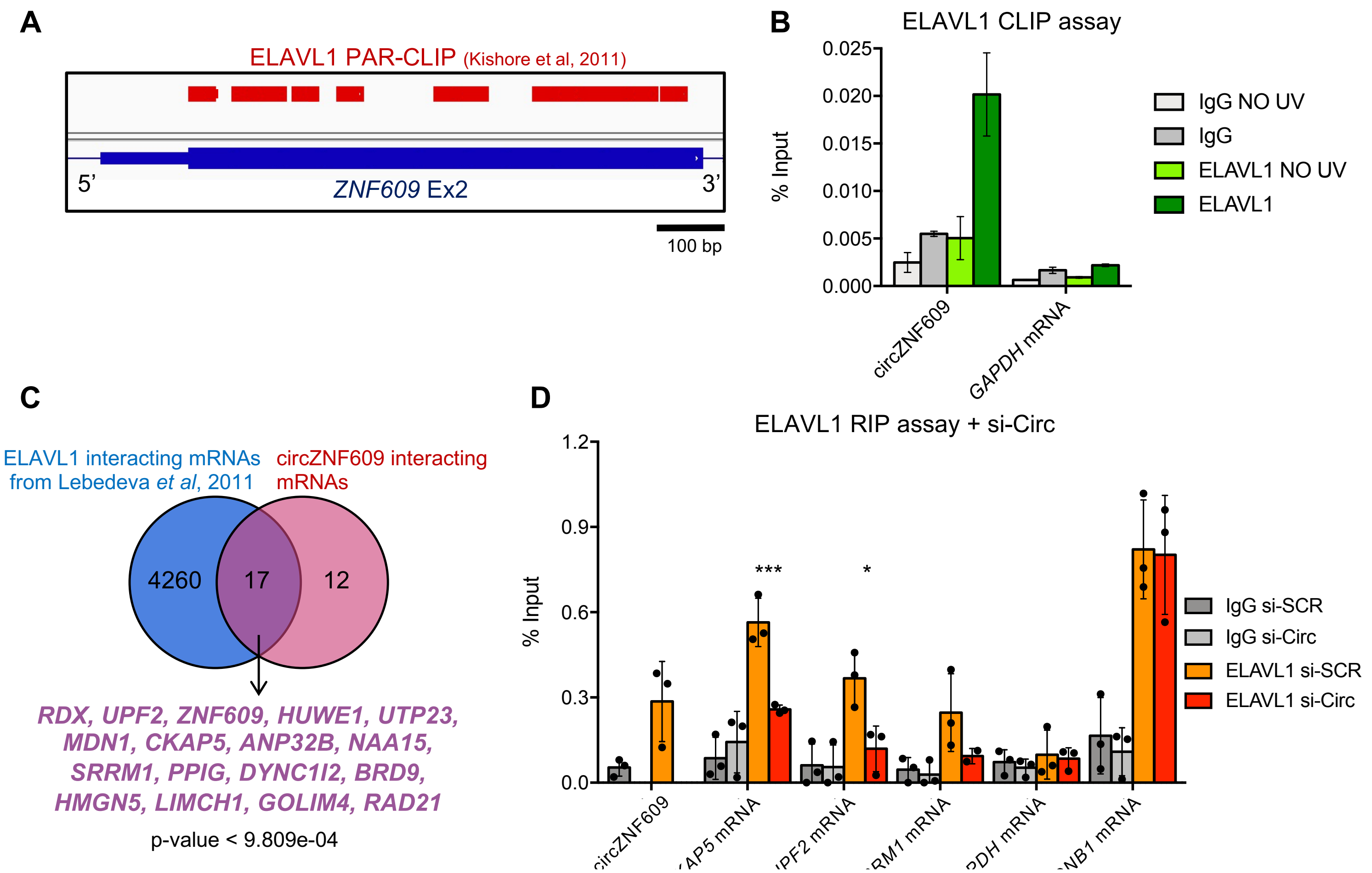


circZNF609 in Rhabdomyosarcoma



circZNF609 in Rhabdomyosarcoma

Figure 2: CircZNF609 interacts with ELAVL1 protein promoting ELAVL1 binding to some of its mRNA interactors



circZNF609 in Rhabdomyosarcoma

Figure 3: CircZNF609 sustains translation and stability of some of its mRNA interactors via ELAVL1

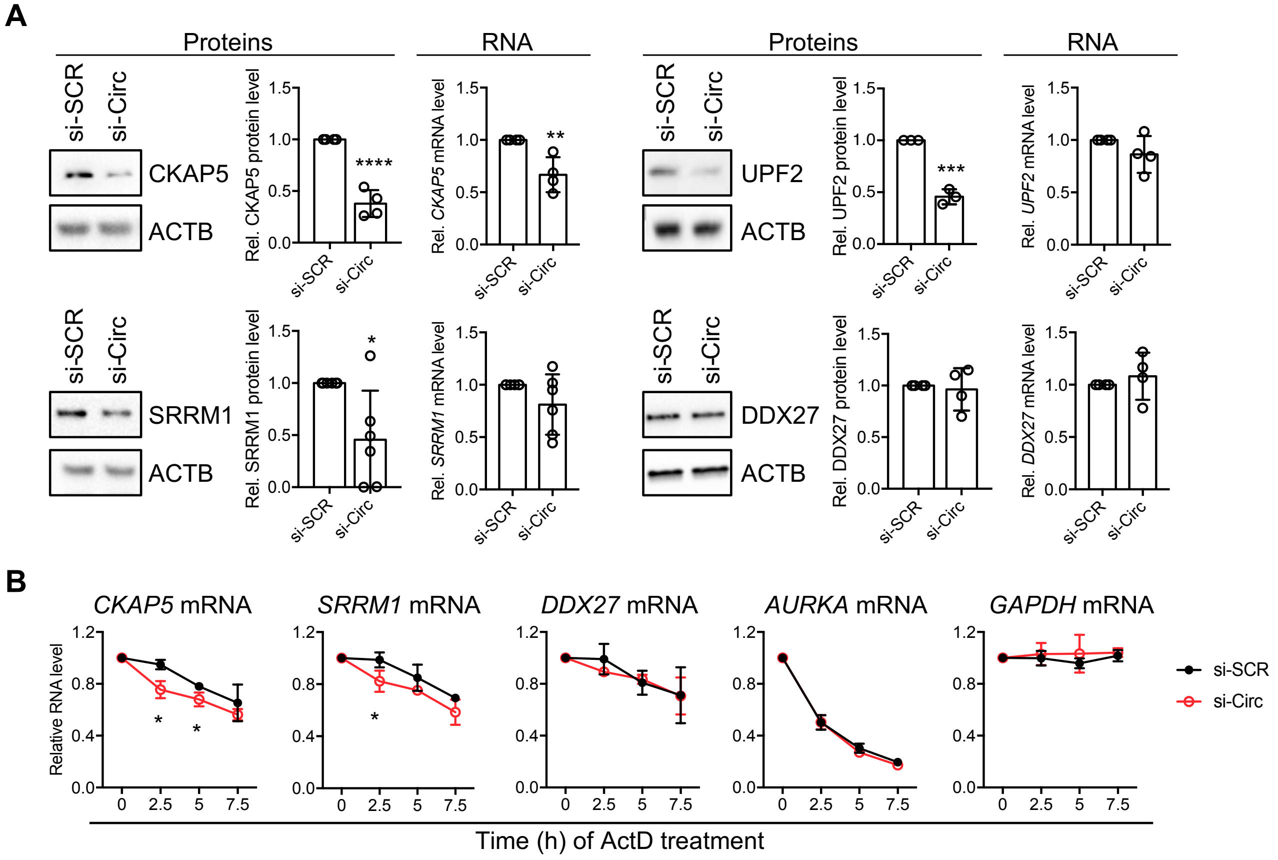
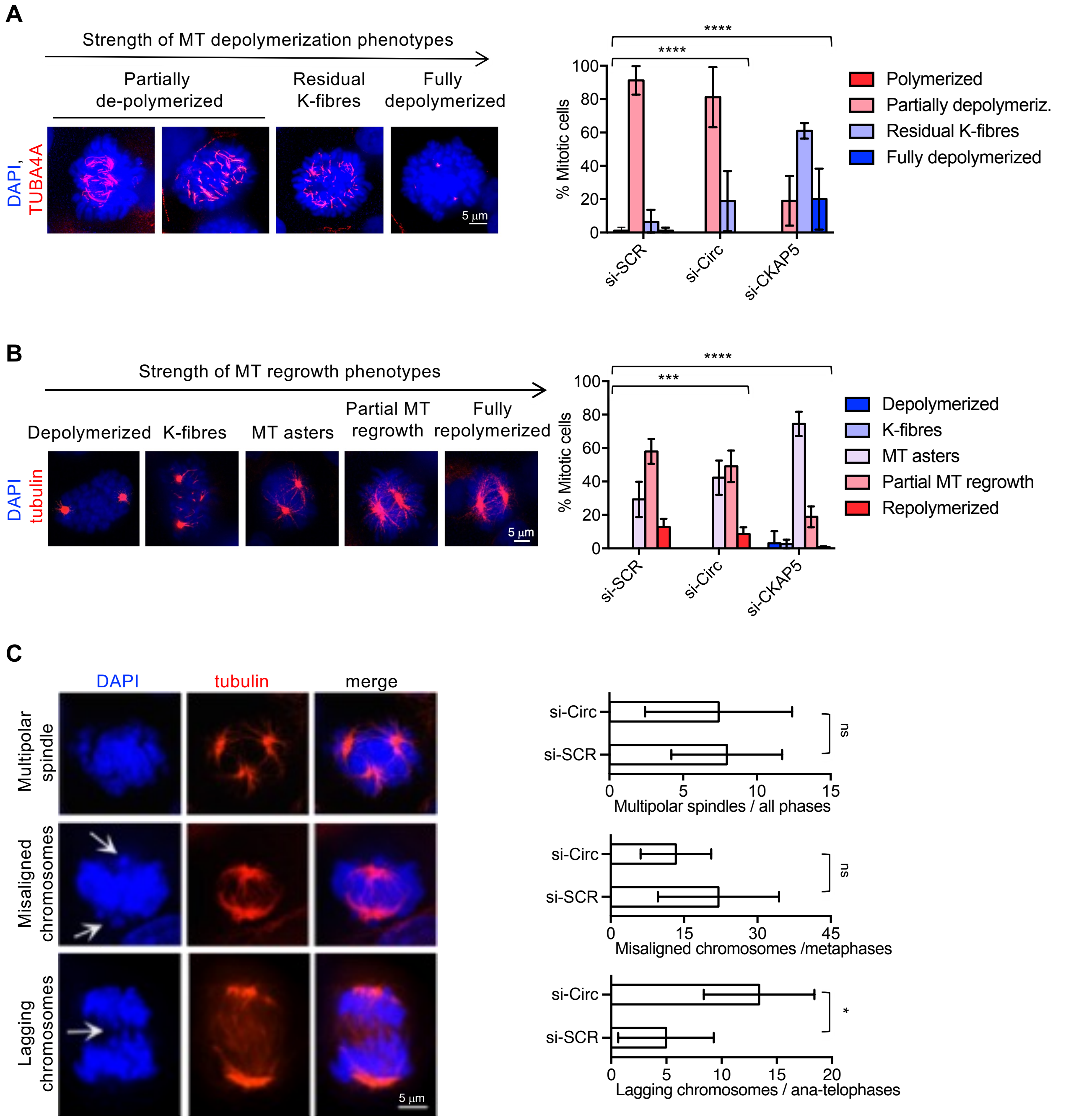
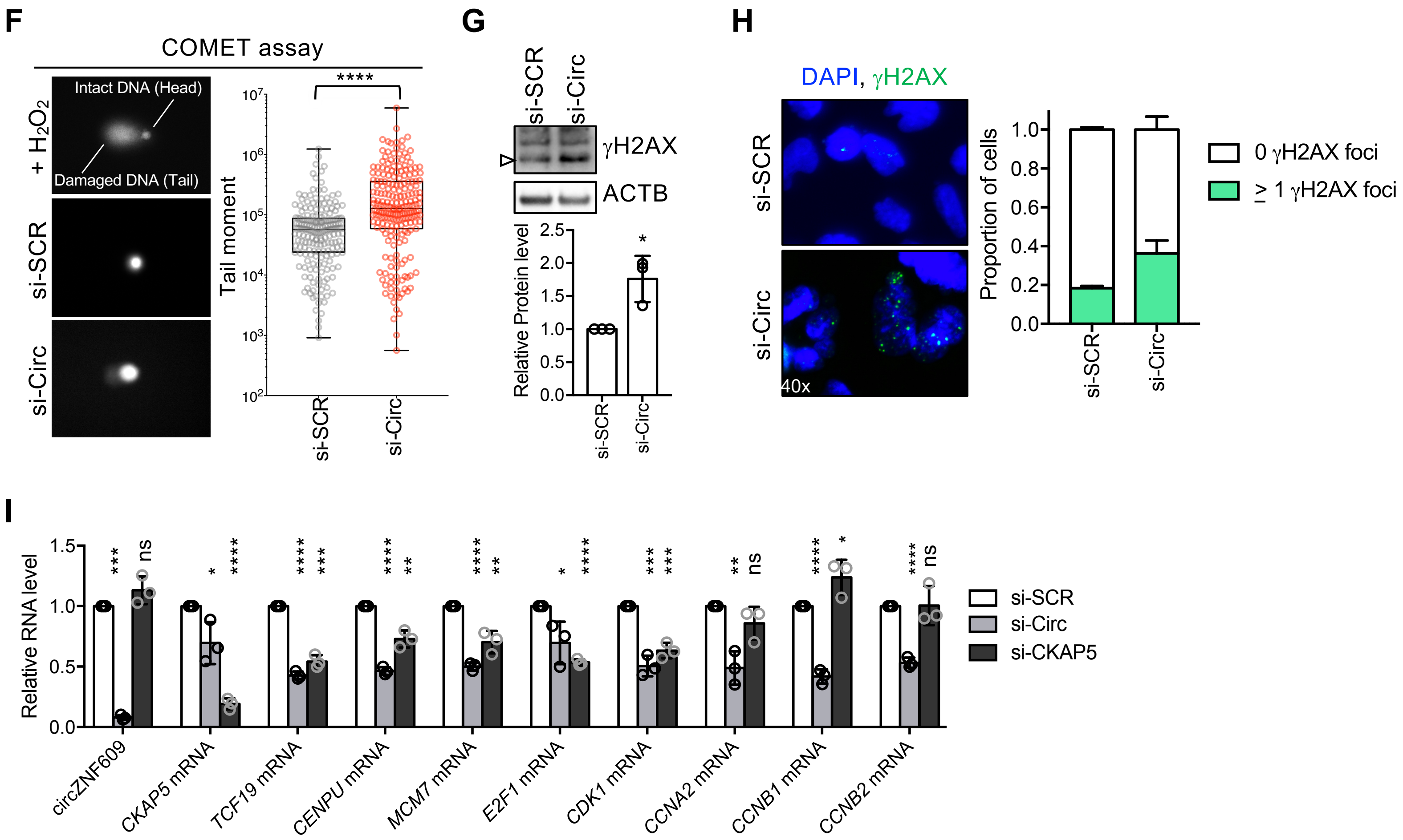


Figure 5: CircZNF609 knock-down destabilises MT cytoskeleton affecting mitotic progression and chromosome segregation

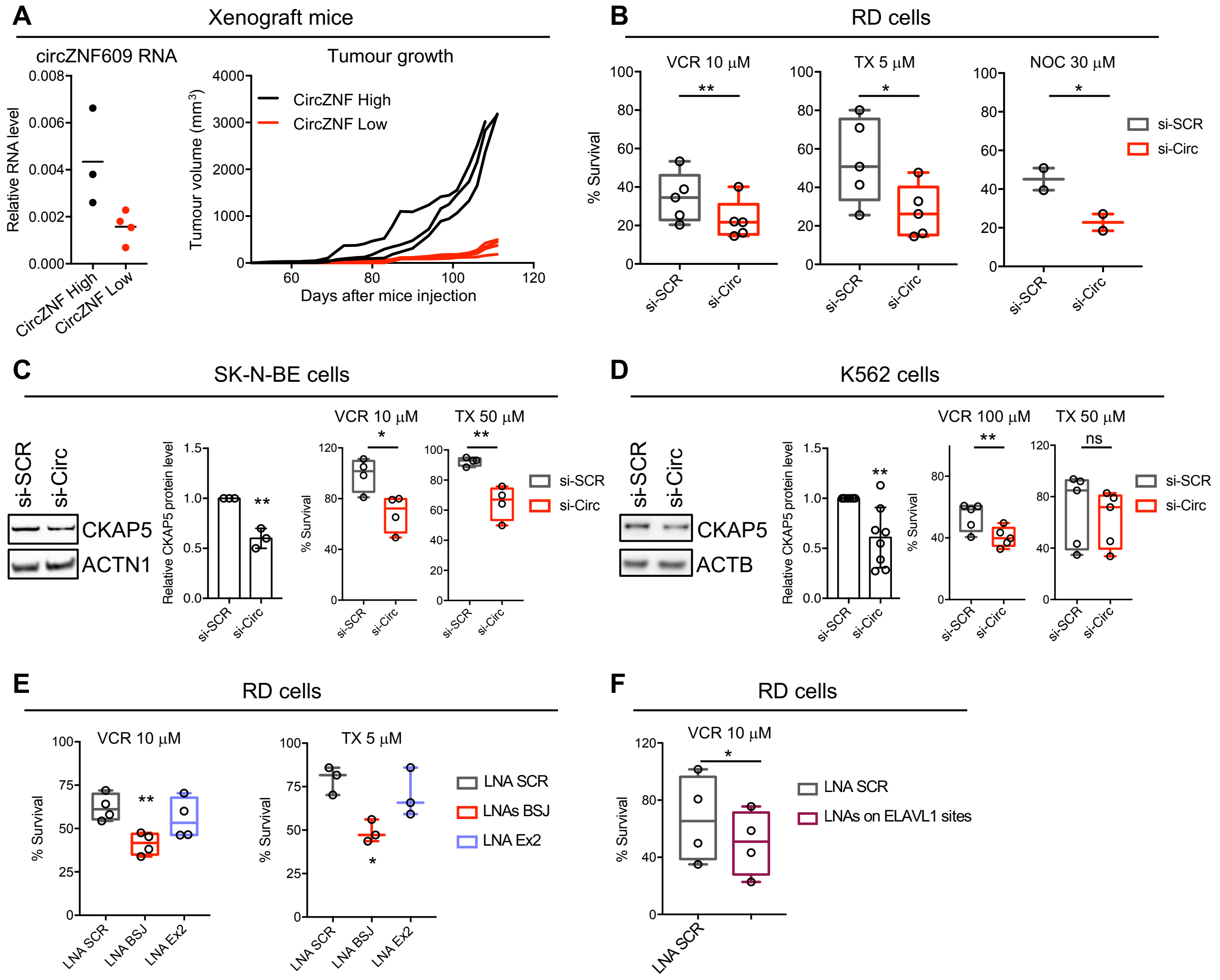


circZNF609 in Rhabdomyosarcoma

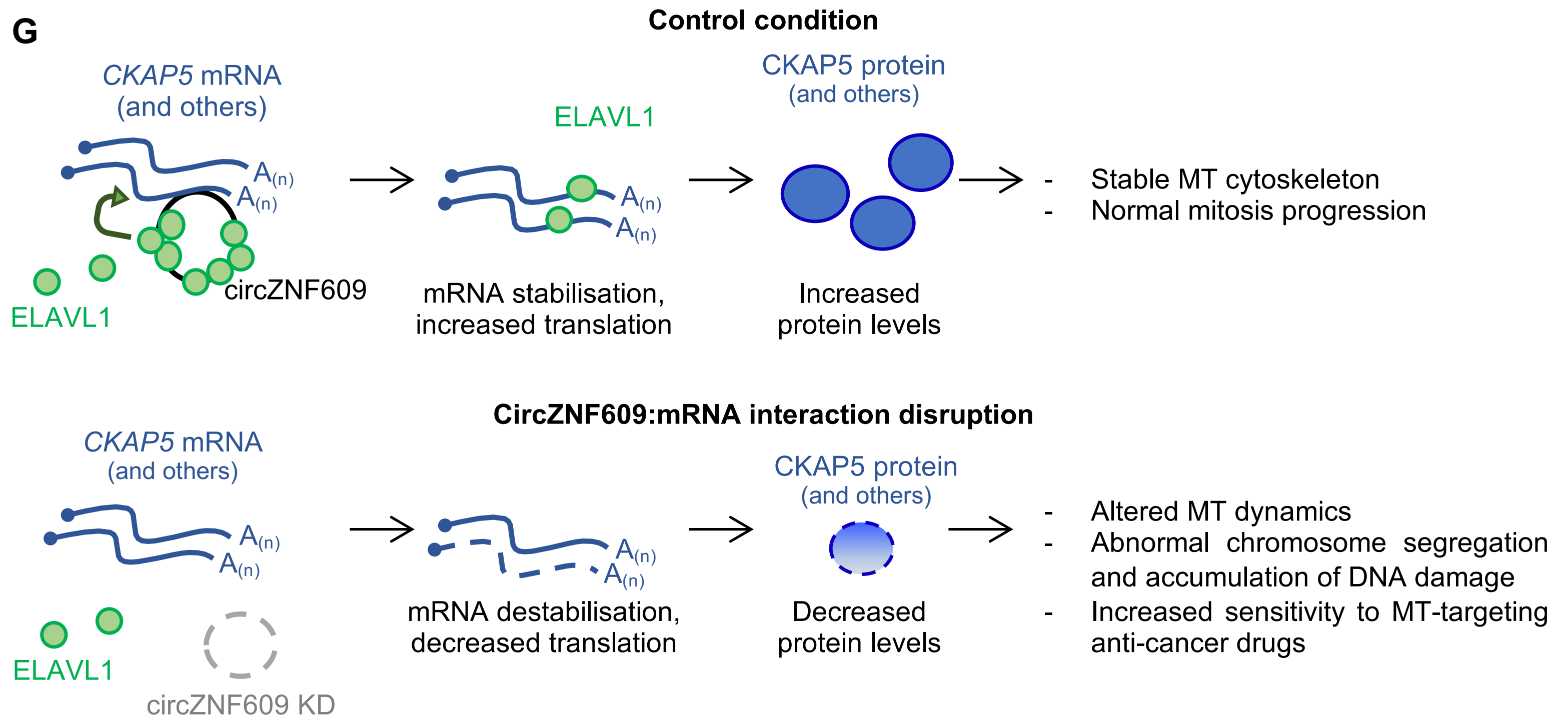


circZNF609 in Rhabdomyosarcoma

Figure 6: Loss of circZNF609/CKAP5 mRNA interaction strengthens the anti-tumour effects of MT-targeting drugs

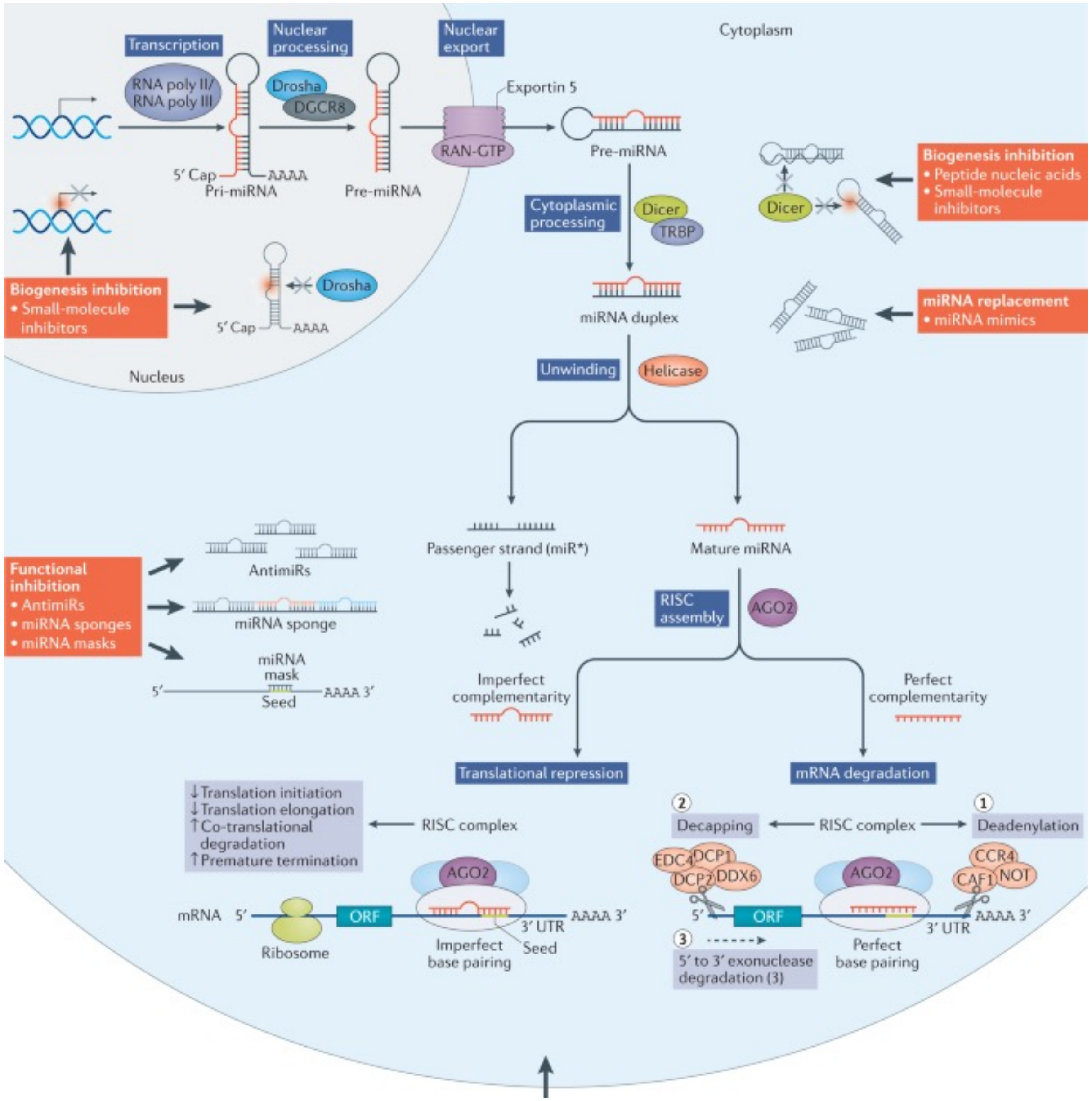


circZNF609 in Rhabdomyosarcoma



Can we use ncRNAs as therapeutic targets?

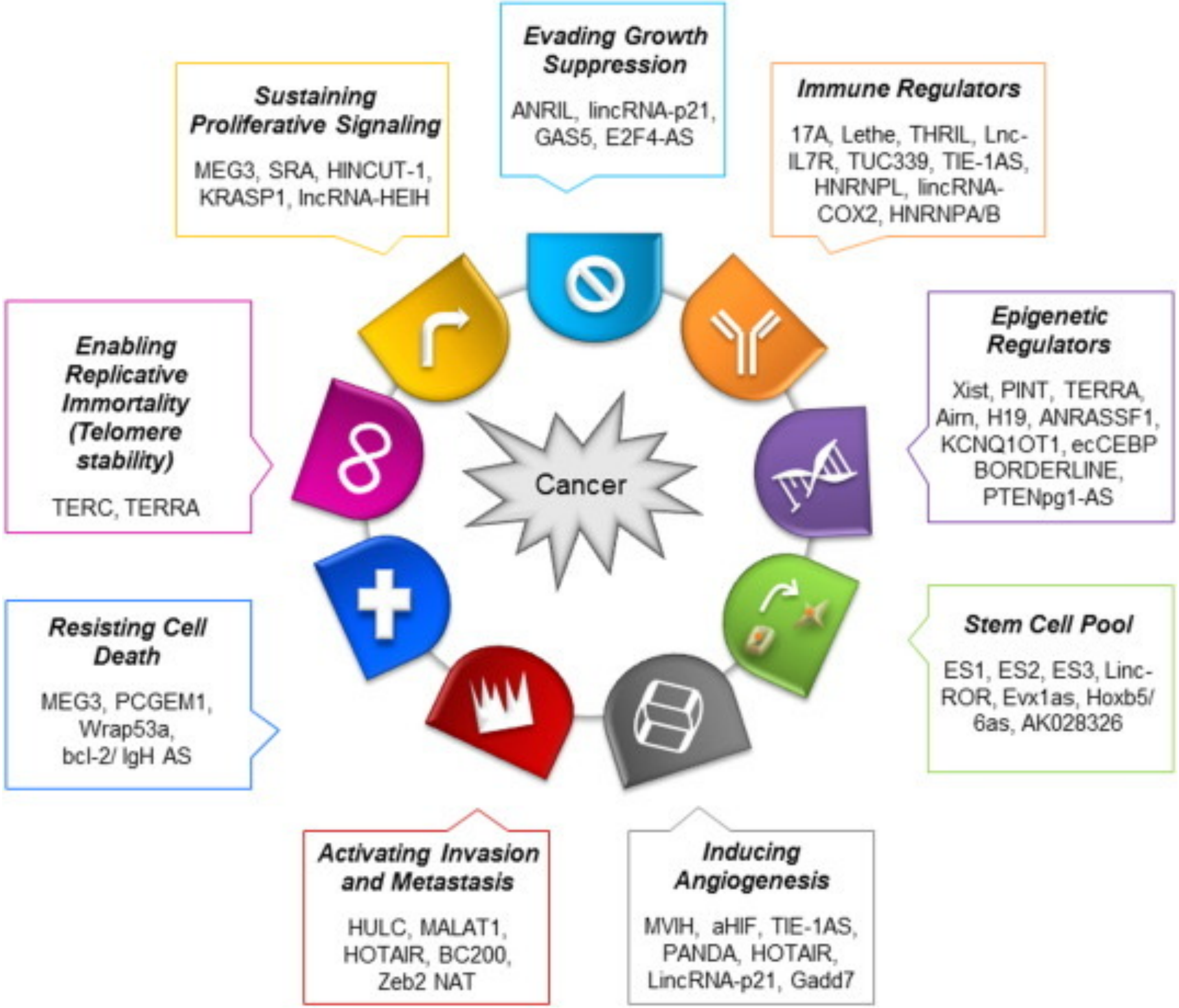
Possible approaches



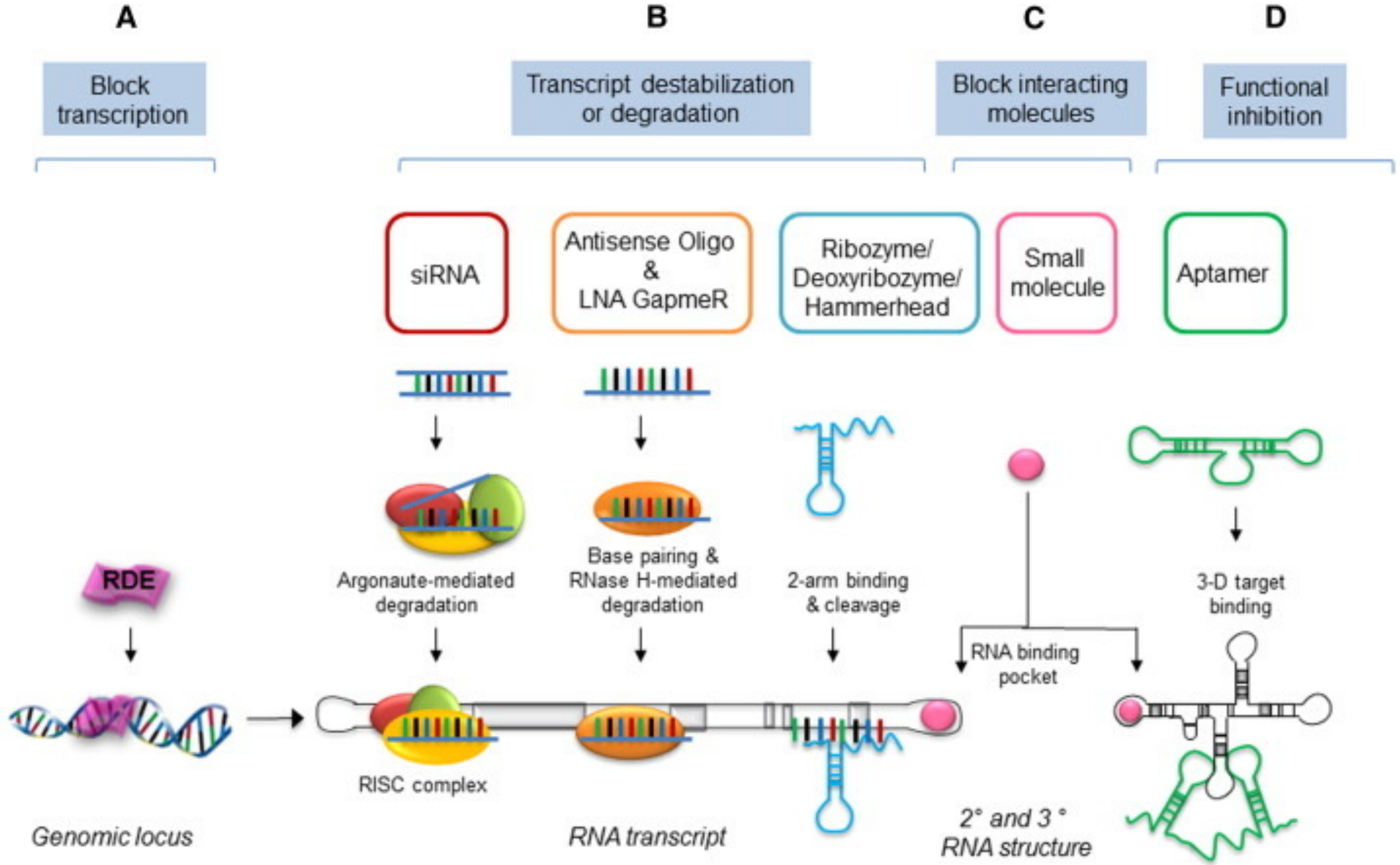
Therapeutic	Type	Modification and delivery	Route of administration	Target organ	Disease	Target gene and pathway	FDA and/or EMA approval year
Fomivirsen (Vitravene)	21-mer ASO	1st gen; PT	Intravitreal	Eye	Cytomegalovirus (CMV) retinitis in immunocompromised patients	CMV IE-2 mRNA	1998 (FDA), 1999 (EMA)[a]
Mipomersen (Kynamro)	20-mer ASO	2nd gen; 2′-MOE gapmer	Subcutaneous	Liver	Homozygous familial hypercholesterolaemia	Apolipoprotein B mRNA	2012 (EMA), 2013 (FDA)
Nusinersen (Spinraza, ASO-10-27)	18-mer ASO	2nd gen; 2′-MOE	Intrathecal	Central nervous system	Spinal muscular atrophy	Survival of motor neuron 2 (*SMN2*) pre-mRNA splicing (exon 7 inclusion)	2017 (EMA), 2016 (FDA)
Eteplirsen (Exondys 51)	30-mer ASO	3rd gen; 2′-MOE PMO	Intravenous	Muscle	Duchenne muscular dystrophy	Dystrophin (*DMD*) pre-mRNA splicing (exon 51 skipping)	2016 (FDA)
Inotersen (Tegsedi, AKCEA-TTR-LRx)	20-mer ASO	2nd gen; 2′-MOE; GalNAc-conjugated	Subcutaneous	Liver	Hereditary transthyretin amyloidosis	Transthyretin (*TTR*) mRNA	2018 (EMA), 2018 (FDA)
Patisiran (Onpattro)	21 nt ds-siRNA	2nd gen; 2′-F/2′-O-Me; liposomal	Intravenous	Liver	Hereditary transthyretin amyloidosis	Transthyretin (*TTR*) mRNA	2018 (EMA), 2019 (FDA)
Golodirsen (Vyondys 53, SRP-4053)	25-mer ASO	3rd gen; 2′-MOE PMO	Intravenous	Muscle	Duchenne muscular dystrophy	*DMD* pre-mRNA splicing (exon 53 skipping)	2019 (FDA)
Givosiran (Givlaari)	21 nt ds-siRNA	2nd gen; 2′-F/2′-O-Me; GalNAc-conjugated	Subcutaneous	Liver	Acute hepatic porphyria	Delta aminolevulinic acid synthase 1 (*ALAS1*) mRNA	2020 (EMA), 2019 (FDA)
Viltolarsen (Viltepso, NS-065, NCNP-01)	21-mer ASO	3rd gen; 2′-MOE PMO	Intravenous	Muscle	Duchenne muscular dystrophy	*DMD* pre-mRNA splicing (exon 53 skipping)	2020 (FDA)
Volanesorsen (Waylivra)	20-mer ASO	2nd gen; 2′-MOE gapmer	Subcutaneous	Liver	Familial chylomicronaemia syndrome	Apolipoprotein CIII (*APOC3*) mRNA	2019 (EMA)
Inclisiran (Leqvio, ALN-PCSsc)	22 nt ds-siRNA	2nd gen; 2′-F/2′-O-Me; GalNAc-conjugated	Subcutaneous	Liver	Atherosclerotic cardiovascular disease, elevated cholesterol, homozygous/heterozygous familial hypercholesterolaemia	Proprotein convertase subtilisin/kexin type 9 (*PCSK9*) mRNA	2020 (EMA)
Lumasiran (Oxlumo, ALN-GO1)	21 nt ds-siRNA	2nd gen; 2′-F/2′-O-Me; GalNAc-conjugated	Subcutaneous	Liver	Primary hyperoxaluria type 1	Hydroxyacid oxidase 1 (*HAO1*) mRNA	2020 (EMA), 2020 (FDA)

ASO, antisense oligonucleotide; ds, double-stranded; GalNAc, *N*-acetylgalactosamine; gen, generation; PMO, phosphoroamidate morpholino oligomer; PT, phosphothiorate; siRNA, small interfering RNA. [a]Marketing was stopped in 2002 after development of potent antiretroviral therapeutics.

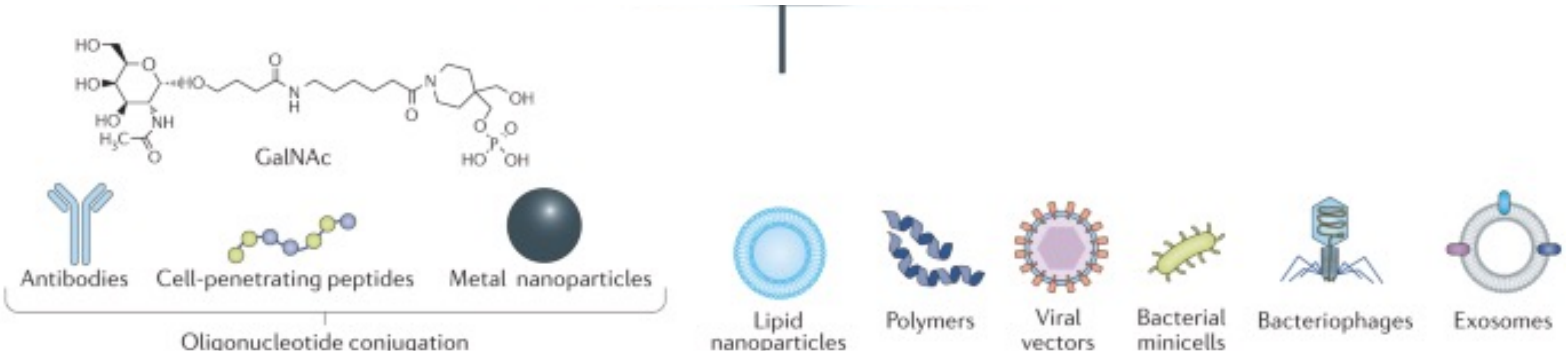
Possible targets



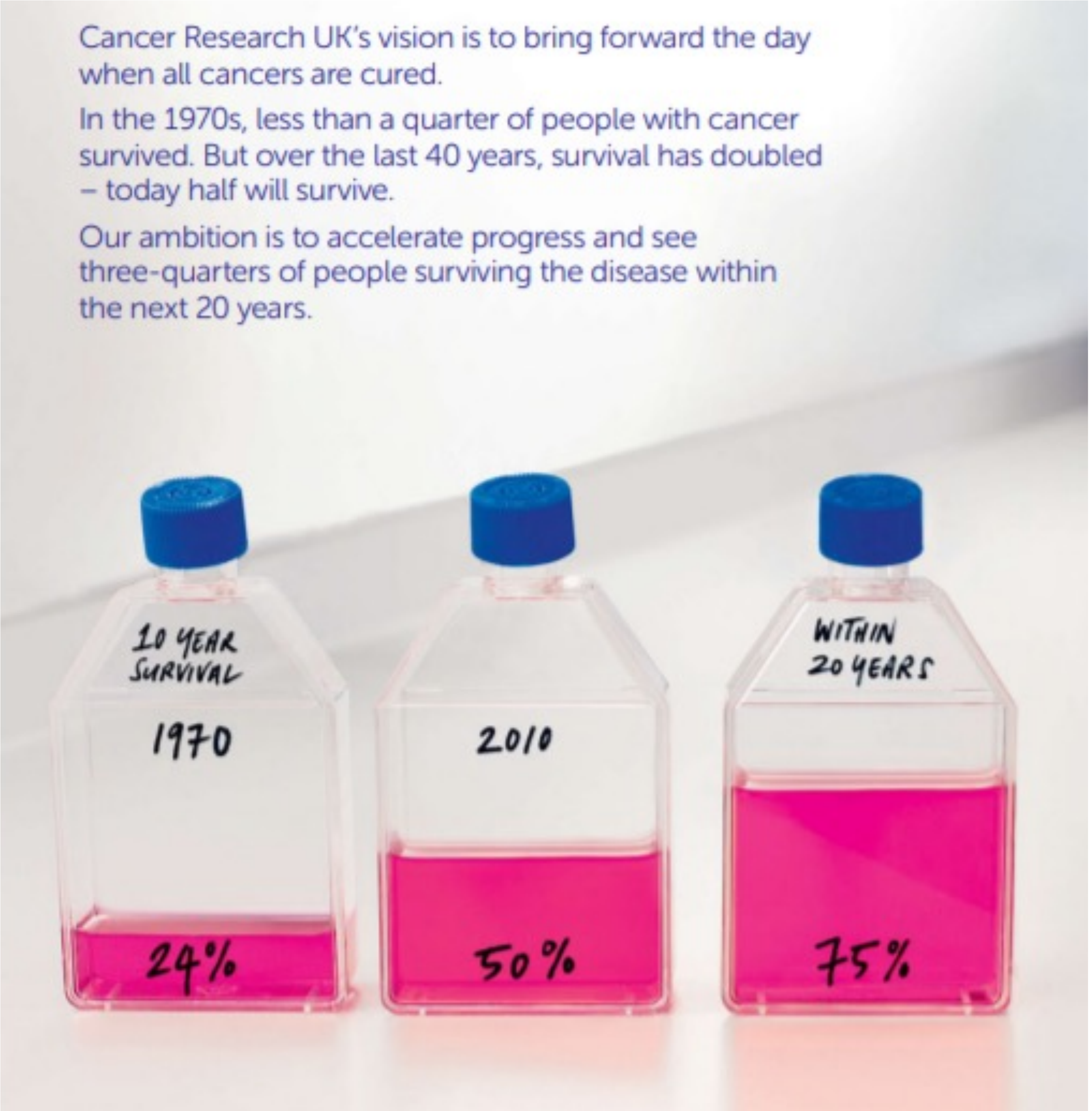
Possible targets



Possible delivery methods



The future


Cancer Research UK's vision is to bring forward the day when all cancers are cured.
In the 1970s, less than a quarter of people with cancer survived. But over the last 40 years, survival has doubled – today half will survive.
Our ambition is to accelerate progress and see three-quarters of people surviving the disease within the next 20 years.
10 YEAR SURVIVAL
1970
24%
2010
50%
WITHIN 20 YEARS
75%

The future

STRATEGY HIGHLIGHTS

DIAGNOSING CANCER EARLIER

Diagnosing cancer early can radically improve the chances of survival. We will substantially increase our investment to support the earlier diagnosis of cancer.

TACKLING CANCERS OF UNMET NEED

Lung, pancreatic, oesophageal cancers and brain tumours have extremely poor survival rates. We will dramatically increase our research effort into these cancers to accelerate progress.

UNDERSTANDING CANCER

We will continue to support crucial research to improve our understanding of what causes and drives cancer.

DEVELOPING NEW TREATMENTS

We will discover and develop new drugs, diagnostics, surgery and radiotherapy techniques – quickening the pace at which research is translated into benefit for patients.

PERSONALISING CANCER TREATMENT

We will optimise every individual's chance of beating cancer by developing personalised approaches to prevention, screening and treatment.

TACKLING TOBACCO TO SAVE LIVES

We will work towards the day when the UK is tobacco-free, in particular by protecting children and by finding more effective ways to help people quit smoking.

CAMPAIGNING FOR THE BEST CANCER SERVICES

We want the UK's survival rates to be among the best in the world. We will campaign for the best public health and cancer services, in all areas of the UK.

ENGAGING PATIENTS IN THE FIGHT AGAINST CANCER

We will give every cancer patient and those close to them the opportunity to join the fight against cancer.

The future

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Q&A:

Bibliography

- Schmitt AM, Chang HY. Long Noncoding RNAs in Cancer Pathways. *Cancer Cell*. 2016;29(4):452-463. doi:10.1016/j.ccell.2016.03.010
- Douglas Hanahan, Robert A Weinberg, The Hallmarks of Cancer, Cell, Volume 100, Issue 1, 2000,Pages 57-70, ISSN 0092-8674.
- Douglas Hanahan, Robert A. Weinberg, Hallmarks of Cancer: The Next Generation, Cell, Volume 144, Issue 5, 2011,Pages 646-674,ISSN 0092-8674,
- Mansi A. Parasramka, Sayantan Maji, Akiko Matsuda, Irene K. Yan, Tushar Patel, Long non-coding RNAs as novel targets for therapy in hepatocellular carcinoma, Pharmacology & Therapeutics, Volume 161,2016, Pages 67-78,ISSN 0163-7258,