Long non-coding RNAs in cancer: then, now and ahead

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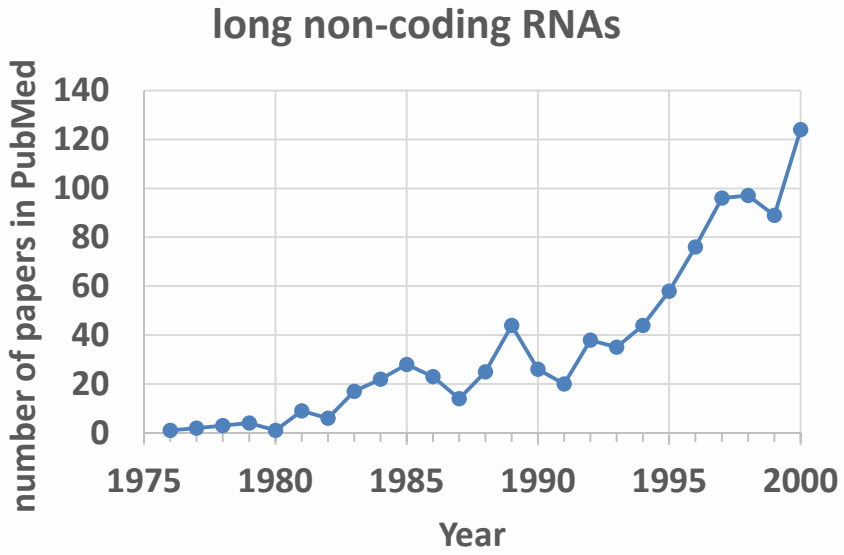
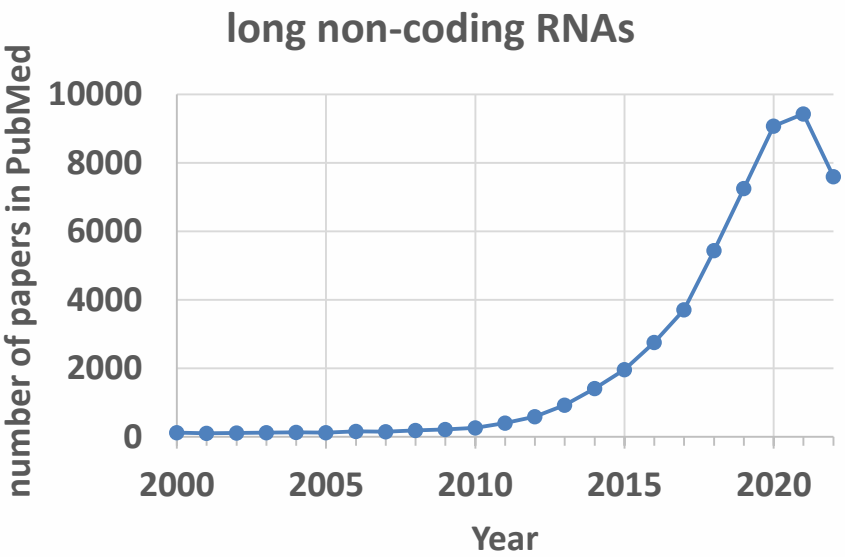
Cancer Genomics

GENOME 20+2

FAPESP 60 YEARS 1962 - 2022

Number of papers per year in PubMed





C.J. Brown et al., Nature 349: 38-44, 1991 ↑
Xist lncRNA = inactive-X specific transcript

↑ Human genome sequence
International Human Genome Sequencing Consortium, *Nature* ***409***, 860 (2001)
Venter J. C., et al., *Science* **291**, 1304 (2001)

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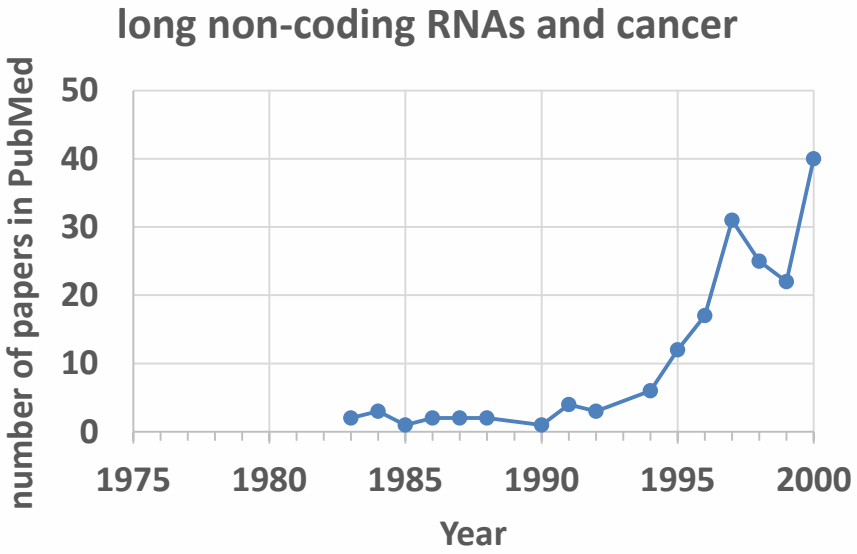
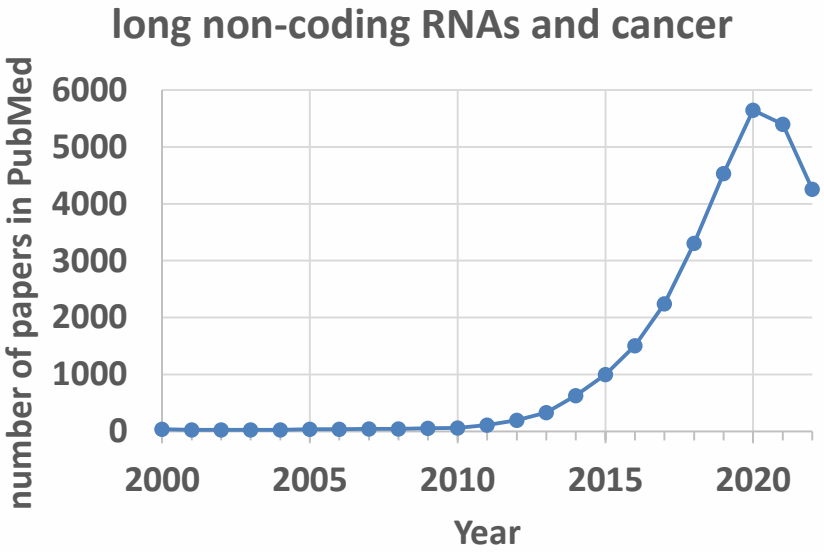
Number of papers per year in PubMed





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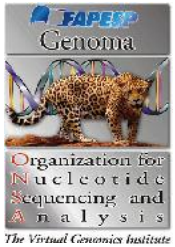
LUDWIG INSTITUTE FOR CANCER RESEARCH

The FAPESP-LICR Human Cancer Genome Project

A Program for Human Gene Discovery and Complete Sequence Compilation



Total (all groups together)

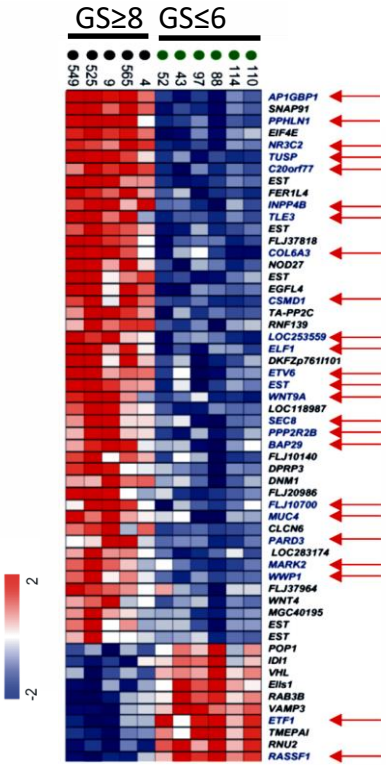
Mitochondria	62,114	(5.34%)
rRNA	44,891	(3.86%)
Bacteria	53,384	(4.59%)
Known Human Genes	203,569	(17.50%)
Unigene Contigs	263,930	(22.69%)
Non-unigene ESTs	94,967	(8.16%)
Paralogs	7,914	(0.68%)
Non-Human Protein	11,080	(0.95%)
ESTs	32	(0.00%)
DNA	126	(0.01%)
Repeats	75,624	(6.50%)
Nomatches	**344,658**	**(29.63%)**
Human Protein	991	(0.09%)
Total number of sequences	**1,163,280**	

Last update: Fri May18 06:00:01 EST 2001

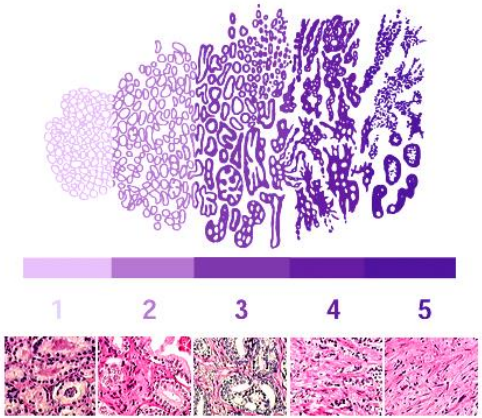
E. Dias-Neto et al., *Proc. Natl. Acad. Sci. USA* 97: 3491-3496, 2000
A. Camargo et al., *Proc. Natl. Acad. Sci. USA* 98: 12103-12108, 2001
H. Brentani et al., *Proc. Natl. Acad. Sci. USA* 100: 13418-13423, 2003

Oncogene (2004) 23, 6684–6692
© 2004 Nature Publishing Group All rights reserved 0950-9232/04 $30.00
www.nature.com/onc

Antisense intronic non-coding RNA levels correlate to the degree of tumor differentiation in prostate cancer



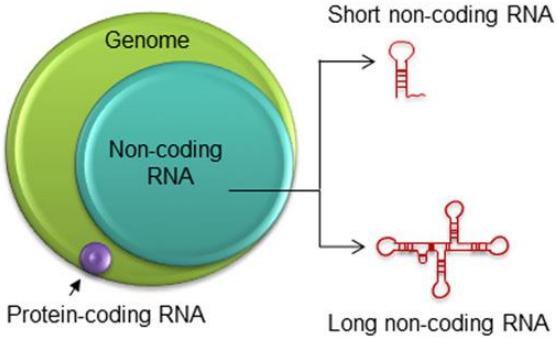
24 out of the 56 genes are antisense non-coding RNAs

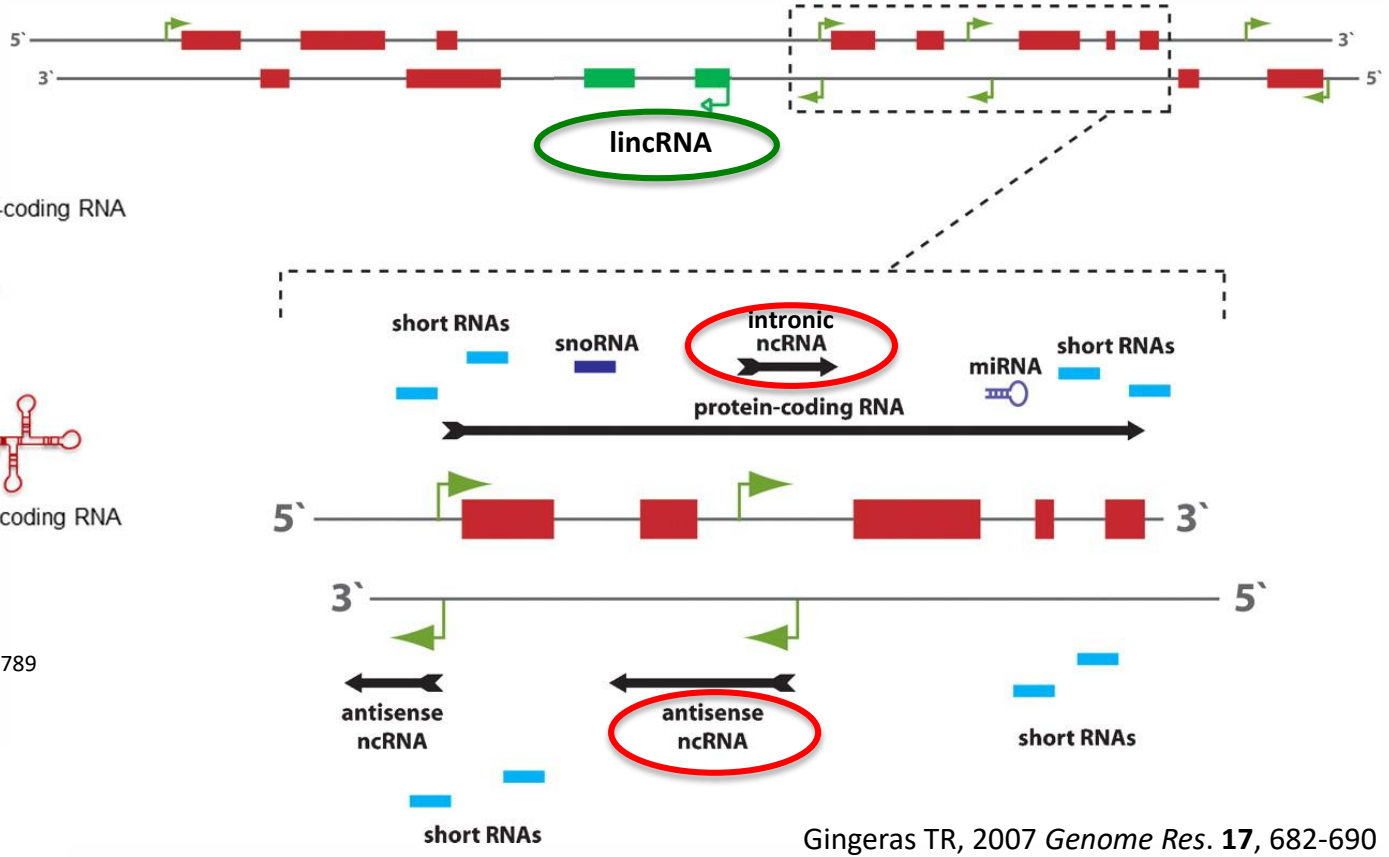


High Gleason Score (GS) ≥ 5 + 3
Low Gleason Score (GS) ≤ 3 + 3

Reis et al., Oncogene (2004) 23: 6684-6692

Identification of the eukaryotic mammalian transcriptome complexity has redefined the concept of a gene





ENCODE – ENCyclopedia Of Dna Elements
Birney et al. 2007 *Nature* **447**, 799-816
Derrien et al., 2012 *Genome Res* **22**, 1775-1789

Nakaya et al., 2007 *Genome Biology* **8**, R43

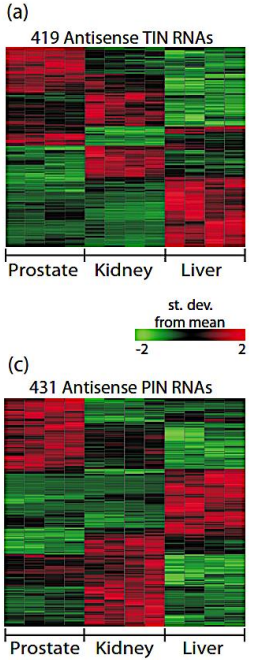
Gingeras TR, 2007 *Genome Res*. **17**, 682-690

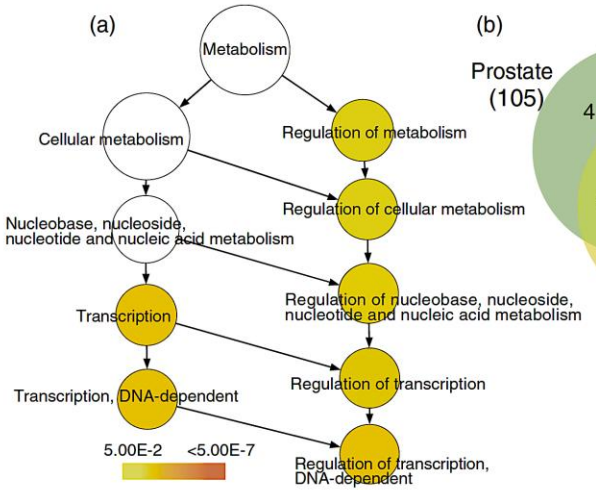
Custom designed oligo-microarray to probe the intronic lncRNA transcriptome in three different human tissue samples

Genome mapping and expression analyses of human intronic noncoding RNAs reveal tissue-specific patterns and enrichment in genes related to regulation of transcription

Helder I Nakaya, Paulo P Amaral, Rodrigo Louro, André Lopes, Angela A Fachel, Yuri B Moreira, Tarik A El-Jundi, Aline M da Silva, Eduardo M Reis and Sergio Verjovski-Almeida

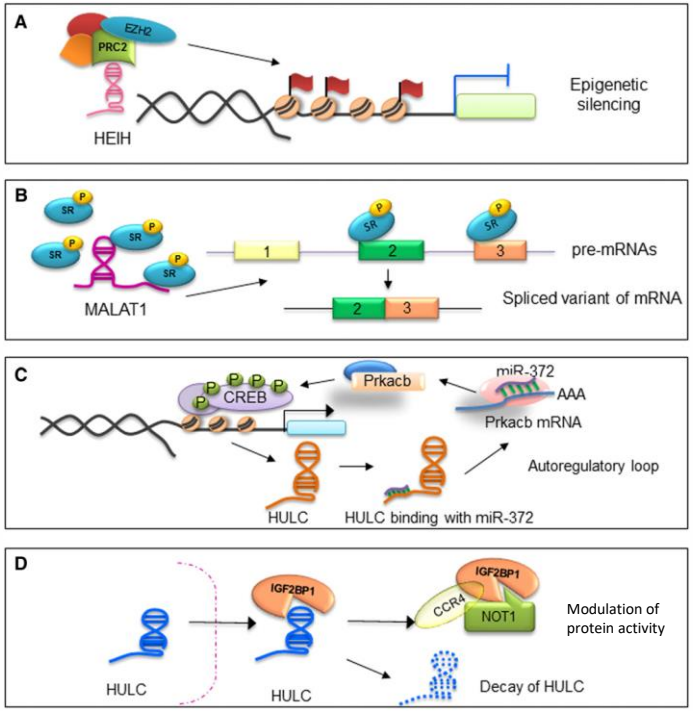
***Genome Biology* 8: R43, 2007**





Most highly expressed intronic lncRNA transcripts map to the loci of protein-coding genes related to regulation of transcription

Examples of mechanisms of lncRNAs associated with cancers



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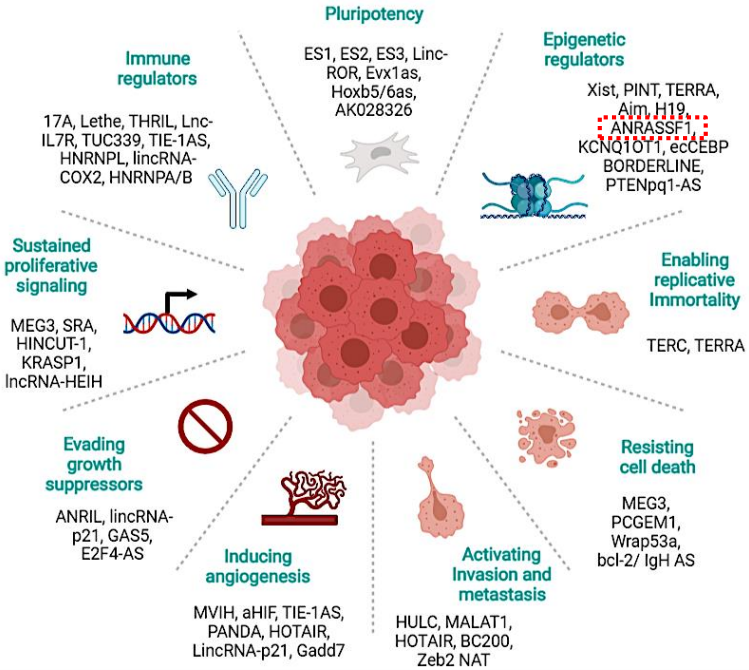
Parasramka et al, Pharmacol Ther 161:67-78, 2016

LncRNAs associated with hallmarks of cancer

LncRNAs contribute to the onset and progression of cancer



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Parasramka et al, Pharmacol Ther 161:67-78, 2016

What are the mechanisms of action of long noncoding RNAs?

ANRASSF1 **– an antisense lncRNA involved in the inhibition of tumor suppressor *RASSF1A* gene expression, thus causing increased cell proliferation**
***PLoS Genetics* 9(8): e1003705, 2013**

HIPSTR **– an antisense lncRNA that is involved in regulation of the developmental program in 8-cell and morula human embryos**
***Scientific Reports* 6: 32753, 2016**

Chromatin landscape distinguishes the Genomic Loci of Hundreds of Androgen-Receptor-Associated LincRNAs From the Loci of Non-associated LincRNAs
***Frontiers in Genetics* 9: 132, 2018**

PVT1 **– *PVT1* lincRNA is an overexpressed oncogene that is associated with AR in LNCaP prostate cancer cells. We provide first evidence that *PVT1* signals a genome-wide transcriptional repressive program of tumor suppressor protein-coding genes in prostate cancer cells**
***Cell Communication and Signaling* 19: 5, 2021**

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PLOS | GENETICS

The Intronic Long Noncoding RNA *ANRASSF1* Recruits PRC2 to the *RASSF1A* Promoter, Reducing the Expression of *RASSF1A* and Increasing Cell Proliferation

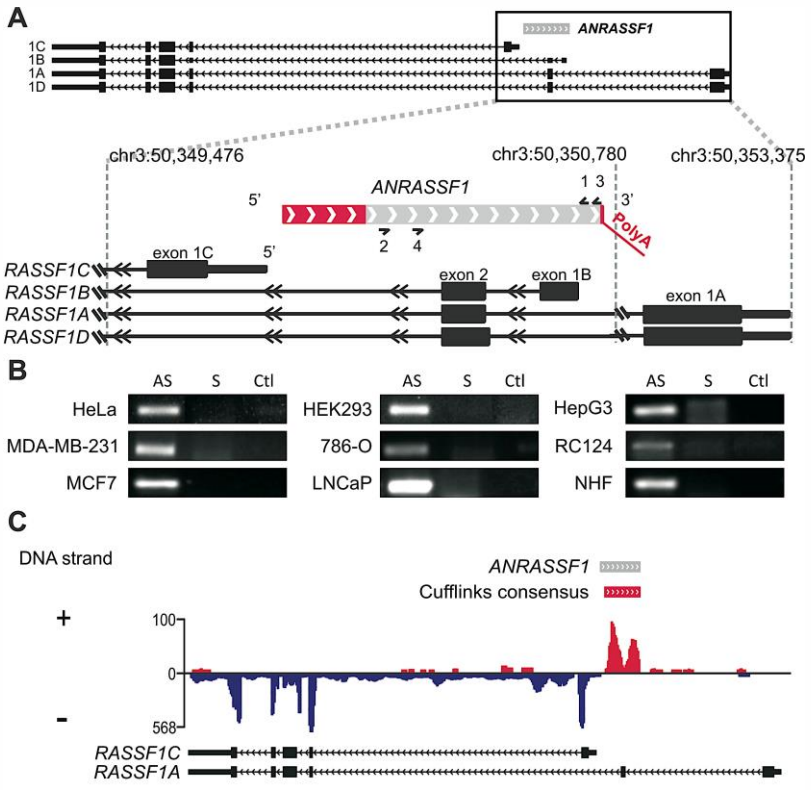
Beckedorff et al. 2013, *PLoS Genetics* 9: e1003705

RASSF1A is an important tumor suppressor gene that is downregulated in prostate cancer.

We identified by RACE, by PCR and by RNA-seq

an unspliced 790 nt lncRNA, expressed in the antisense direction in the locus of *RASSF1*,

We named the antisense lncRNA as *ANRASSF1*



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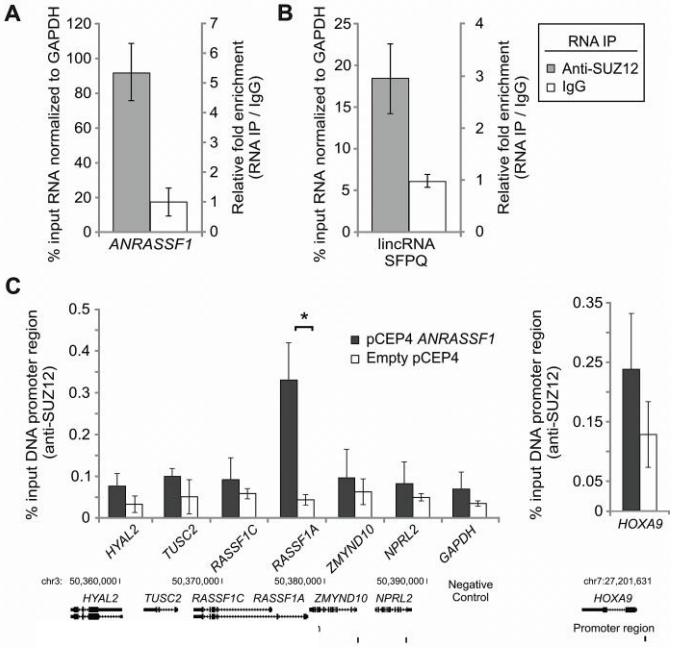
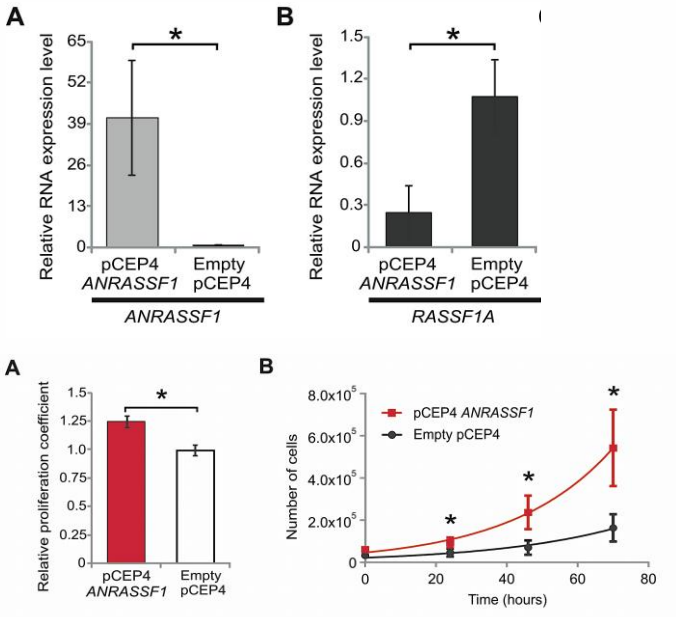
Beckedorff et al. 2013, *PLoS Genetics* 9: e1003705

***ANRASSF1* lncRNA interacts with PRC2 complex**

and recruits PRC2 complex to the *RASSF1A* promoter



***ANRASSF1* lncRNA overexpression inhibits tumor suppressor *RASSF1A* gene expression, thus causing increased cell proliferation**



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SCIENTIFIC REPORTS

Scientific Reports 2016, Sep 8; 6: 32753

OPEN

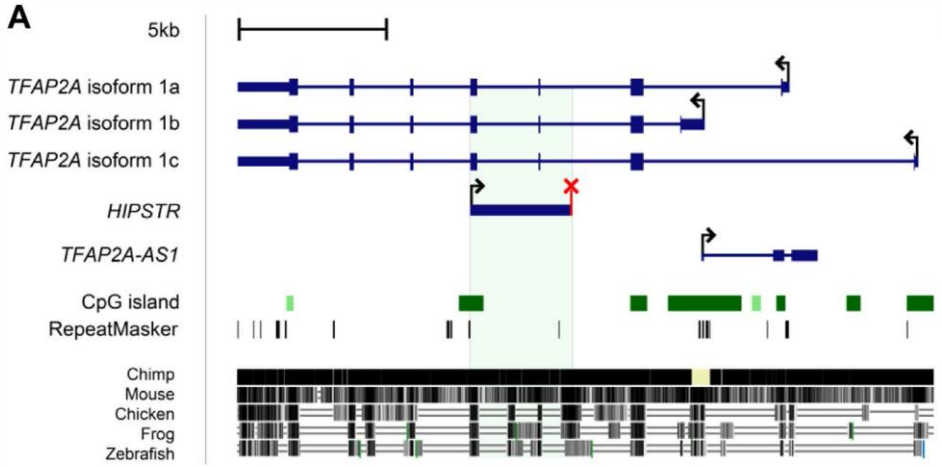
HIPSTR and thousands of lncRNAs are heterogeneously expressed in human embryos, primordial germ cells and stable cell lines

Received: 04 July 2016
Accepted: 11 August 2016
Published: 08 September 2016

Dinar Yunusov[1,2], Leticia Anderson[1,2], Lucas Ferreira DaSilva[1,2], Joanna Wysocka[3], Toshihiko Ezashi[4], R. Michael Roberts[4,5] & Sergio Verjovski-Almeida[1,2]

TFAP2A encodes a TF known to be involved in various cancers including prostate cancer, where *TFAP2A* is downregulated

HIPSTR lncRNA is expressed from chr6:10404735–10408161 in the locus of protein-coding *TFAP2A* gene, in the opposite genomic strand



www.nature.com/scientificreports

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Scientific Reports 2016, Sep 8; 6: 32753

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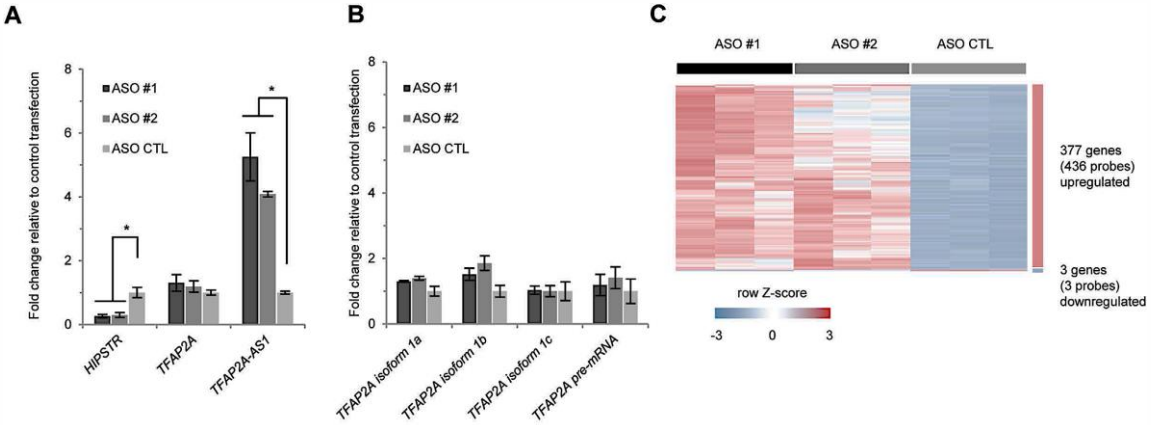
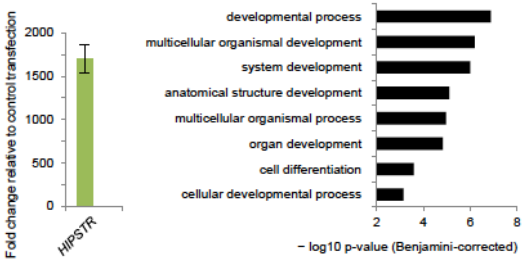
HIPSTR knockdown in HEK293 resulted in genome-wide differential expression of 380 genes located outside of the *TFAP2A* locus, of which 377 (~99.2%) were upregulated.



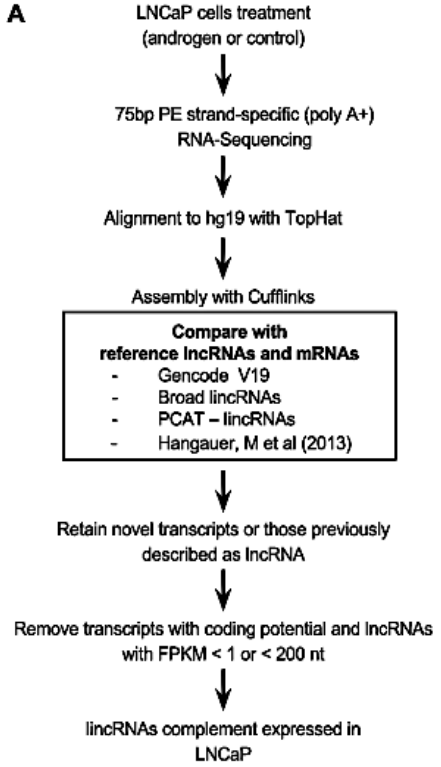
Gene Ontology (GO) analysis of these genes revealed their enrichment in "Developmental Process" and "Cell Differentiation" categories. These results suggest a repressive function for *HIPSTR* in HEK293 cells.

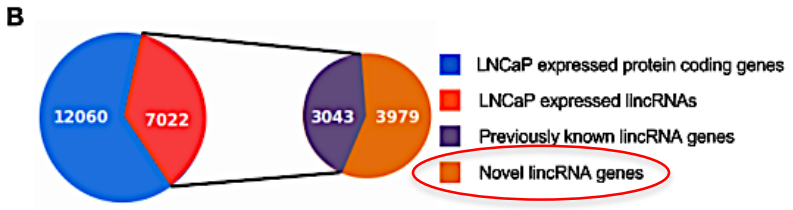


frontiers in Genetics

Chromatin Landscape Distinguishes the Genomic Loci of Hundreds of Androgen-Receptor-Associated LincRNAs From the Loci of Non-associated LincRNAs

daSilva et al., *Frontiers in Genetics* 2018, Apr 25; 9: 132





What is the lincRNA complement of prostate cancer cells?

LNCaP cells treated for 24 h with 1 nM R1881 androgen or vehicle.

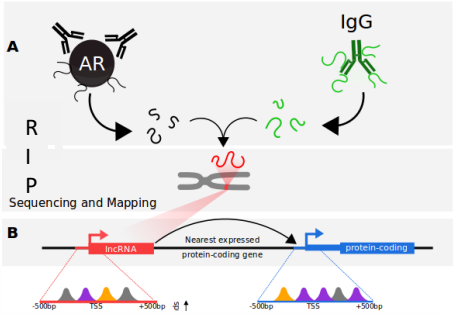
Poly(A)+RNA was extracted.

Stranded RNA libraries were prepared, and RNA-seq was obtained from two samples per LNCaP treatment: ~ 85 million mate pair reads per each of four samples, a total of 340 million paired-end reads.

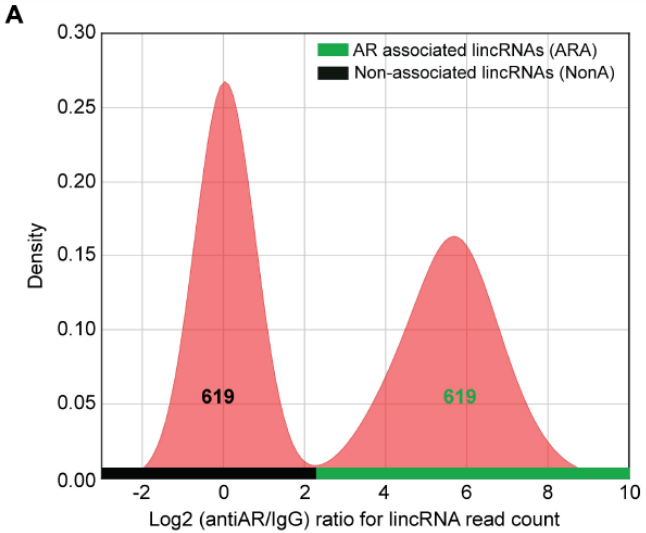
frontiers in Genetics

Chromatin Landscape Distinguishes the Genomic Loci of Hundreds of Androgen-Receptor-Associated LincRNAs From the Loci of Non-associated LincRNAs

daSilva et al., *Frontiers in Genetics* 2018, Apr 25; 9: 132



A large number of lincRNAs are physically associated to the androgen receptor (AR)



RIP-Sequencing of lincRNAs co-immunoprecipitated with anti-AR antibody shows 619 lincRNAs (green) significantly enriched in the anti-AR fraction over IgG control

(FDR < 5% in DESEQ2)

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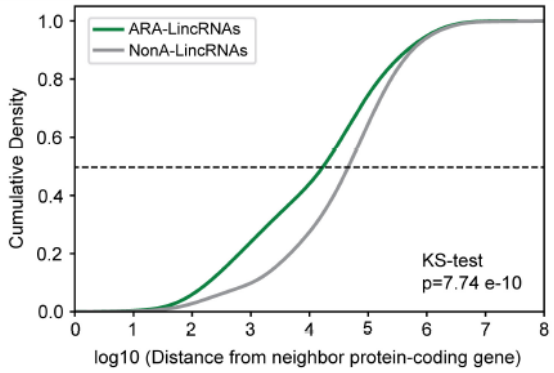
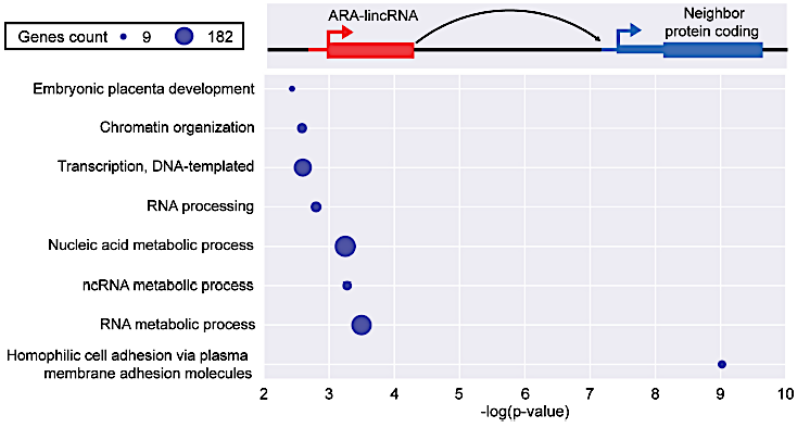
Chromatin Landscape Distinguishes the Genomic Loci of Hundreds of Androgen-Receptor-Associated LincRNAs From the Loci of Non-associated LincRNAs

daSilva et al., *Frontiers in Genetics* 2018, Apr 25; 9: 132

ARA-lincRNAs
map closer to the neighbor protein-coding gene
than the NonA-lincRNAs



Protein coding genes in the vicinity of lincRNAs associated with AR
are enriched in a set of biological functions



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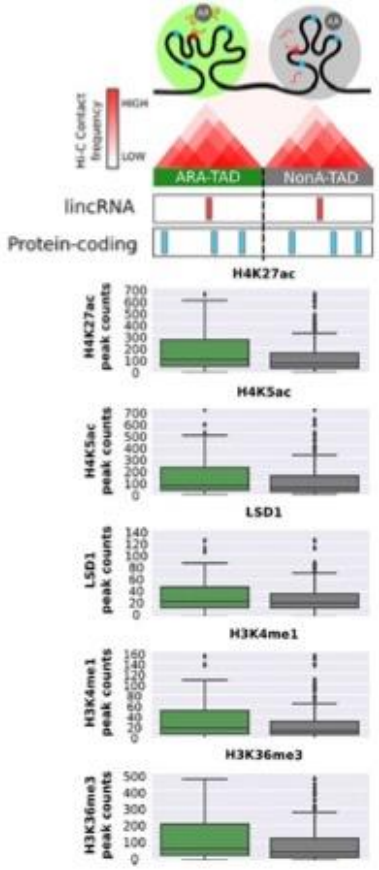
daSilva et al., *Frontiers in Genetics* 2018, Apr 25; 9: 132

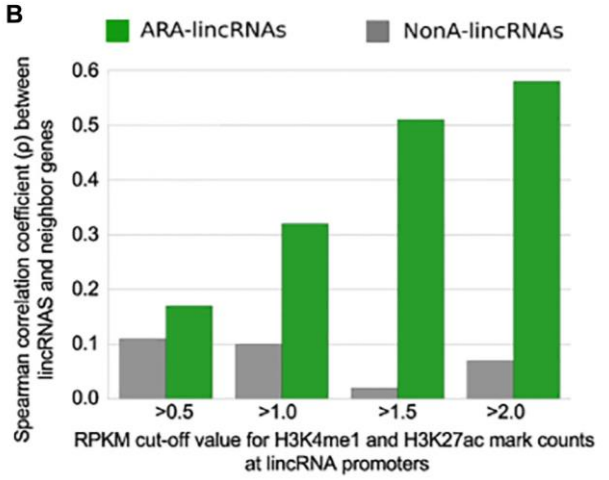
Chromatin topologically associating domains (TADs) of LNCaP that contain ARA-lincRNAs have a significantly higher content of a given set of histone marks, compared with TADs whose lincRNAs are not associated to AR

The identified high-ranked marks suggest that ARA-lincRNAs act as enhancer RNAs

All possible pairwise comparisons resulted in statistically significant differences (p-value < 0.05, t-test)



ARA-lincRNA expression correlates with that of the protein-coding gene neighbor



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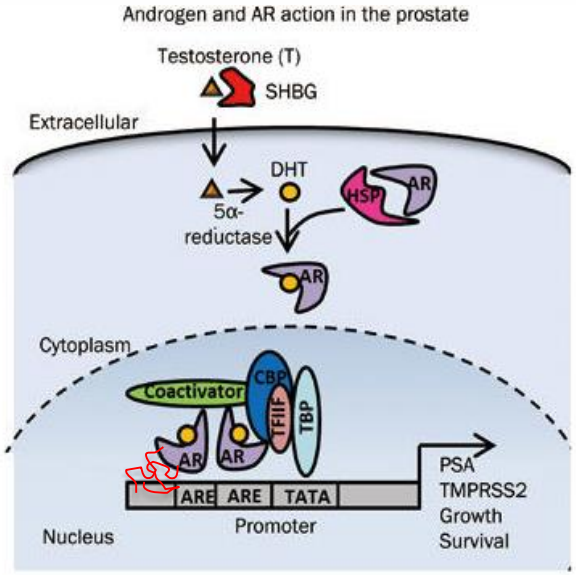


Modified from Tan M.H.E. et al. Acta Pharm. Sinica (2015) 36: 3–23

Perspective

Hundreds of lincRNAs were identified that interact with the Androgen Receptor (AR) complex, and they are candidates to regulate the expression of protein-coding genes either in their vicinity or in the TAD where the lincRNA is expressed.

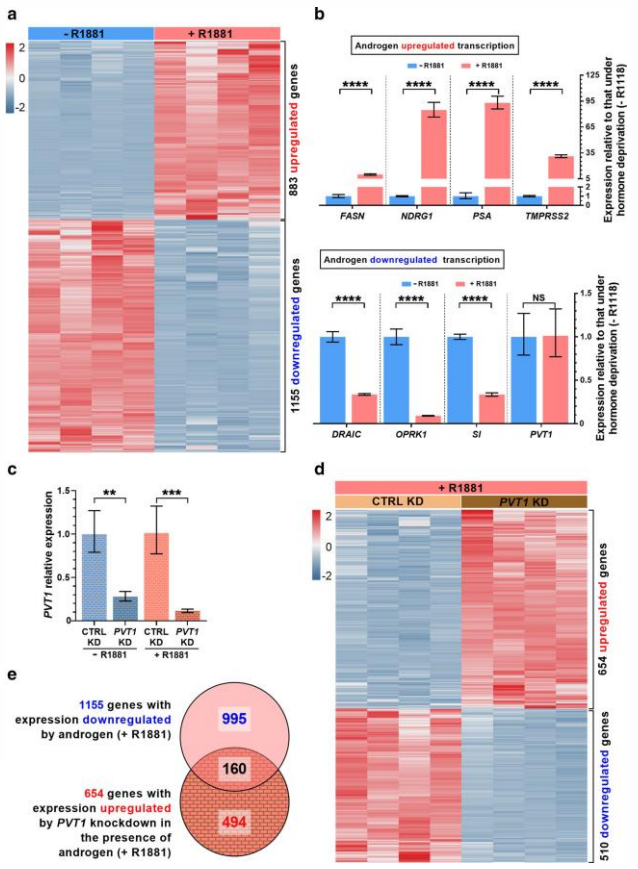
A number of interesting protein-coding genes known to be involved in prostate cancer were identified that have an ARA-lincRNA expressed in their vicinity.

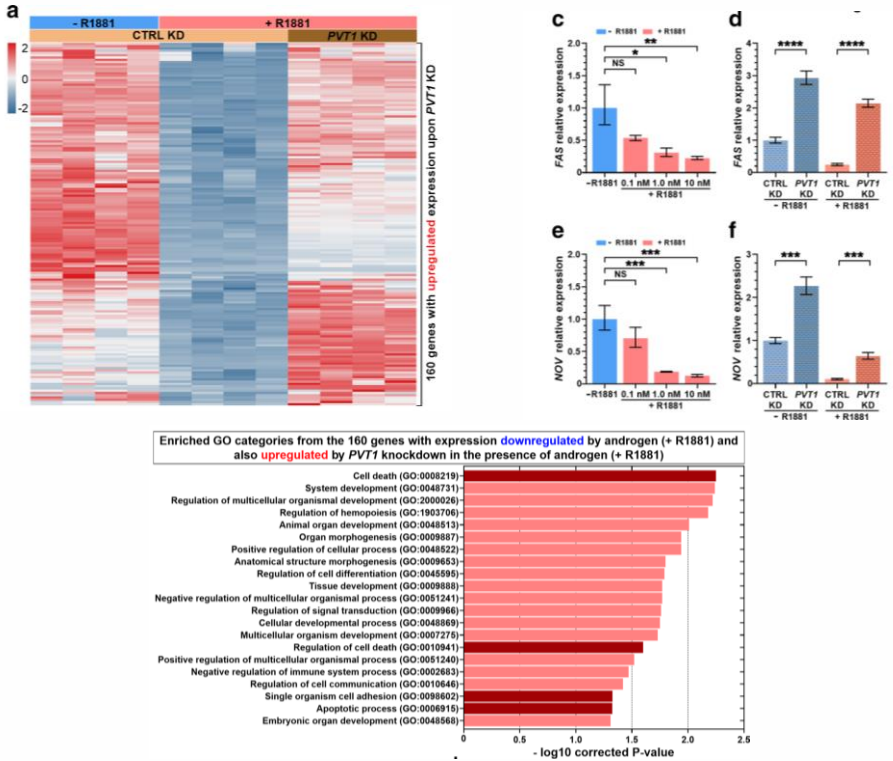
These pairs are candidates for further studies on the possible mechanism of action of the lincRNA on the expression of the protein-coding gene neighbor.

PVT1 lincRNA signals an androgen-dependent transcriptional repression program in prostate cancer cells

Videira et al., Cell Commun Signal (2021) 19: 5

Genes de-repressed by *PVT1* lincRNA knockdown in androgen-stimulated LNCaP cells are enriched in tumor suppressor functions

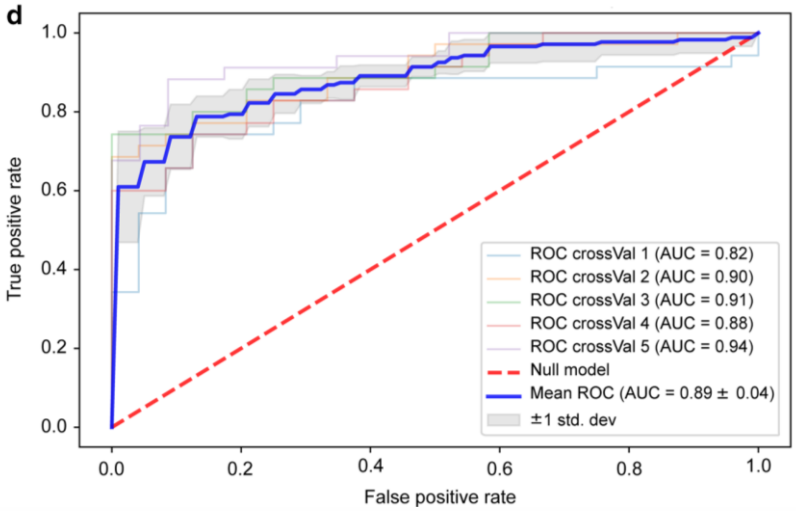


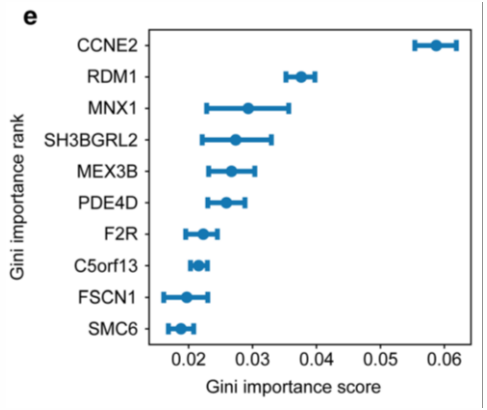


PVT1 lincRNA signals an androgen-dependent transcriptional repression program in prostate cancer cells

Videira et al., Cell Commun Signal (2021) 19: 5

The expression levels of 121 genes down-regulated by *PVT1* lincRNA, identified in our analysis, can predict high-risk Prostate Adenocarcinoma tumors in the TCGA database using a Random Forest machine-learning model (ROC AUC = 0.89).





We show that *PVT1* lincRNA signals a genome-wide transcriptional repression of protein-coding genes in prostate cancer.

The repressed gene set is enriched in tumor suppressor functions, which may lie behind the known aggressive phenotype of tumors expressing high levels of PVT1 lincRNA oncogene.

ASO and siRNA therapeutics that are approved by the FDA as of 2022

ASO and siRNA therapeutics that approved by the FDA.

Type	Drug	Mechanism	Target organ	Disease	Administration
ASO	Nusinersen (Spinraza, ASO-10-27)	Antisense, splicing modulation	Central nervous system	Spinal muscular atrophy	Intrathecal
	Fomivirsen (Vitravene)	Antisense	Eye	Cytomegalovirus Retinitis	Intravitreal
	Eteplirsen (Exondys 51)	Antisense, splicing modulation	Muscle	Duchenne muscular dystrophy	Intravenous
	Golodirsen (Vyondys 53, SRP-4053)	Antisense, splicing modulation	Muscle	Duchenne muscular dystrophy	Intravenous
	Viltolarsen (Viltepso, NS-065, NCNP-01)	Antisense, splicing modulation	Muscle	Duchenne muscular dystrophy	Intravenous
	Casimersen (Amondys 45)	Antisense, splicing modulation	Muscle	Duchenne muscular dystrophy	Intravenous
	Mipomersen (Kynamro)	Antisense	Liver	Familial hypercholesterolaemia	Subcutaneous
	Inotersen (Tegsedi)	Antisense	Liver	Hereditary transthyretin amyloidosis	Subcutaneous
	Volanesorsen (Waylivra)	Antisense	Liver	Familial chylomicronaemia syndrome	Subcutaneous
siRNA	Patisiran (Onpattro)	RNA interference	Liver	Hereditary transthyretin amyloidosis	Intravenous
	Givosiran (Givlaari)	RNA interference	Liver	Acute hepatic porphyria	Subcutaneous
	Inclisiran (Leqvio, ALN-PCSsc)	RNA interference	Liver	Primary hypercholesterolaemia	Subcutaneous
	Lumasiran (Oxlumo, ALN-GO1)	RNA interference	Liver	Primary hyperoxaluria	Subcutaneous

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Sergio Verjovski-Almeida
Maria Gabriela Berzoti Coelho
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Fabio Nunes de Mello
Thalles Souza Lopes
David A. Morales-Vicente
Alexandre Videira

Murilo S. Amaral
Gilbert O. Silveira
Daisy Woellner Santos
Ana Carolina Tahira
João V. M. Malvezzi
Vinicius Mesel

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FAPESP

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