

False positives in microRNA target identification: implications in molecular oncology

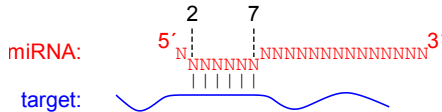
Hervé Seitz

June 13, 2023

This slideshow is available at:

<http://www.igh.cnrs.fr/equip/Seitz/RNA0cc23.pdf>

miRNA target prediction



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

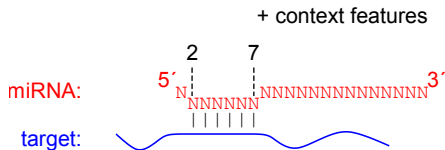
Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

miRNA target prediction



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

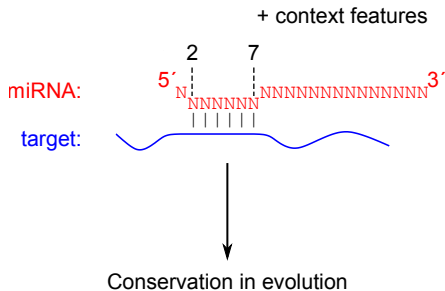
Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

miRNA target prediction



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

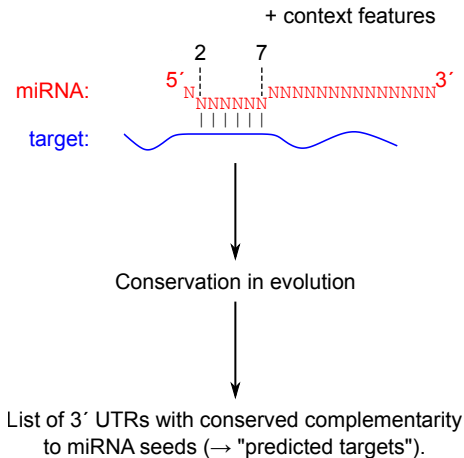
Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

miRNA target prediction



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

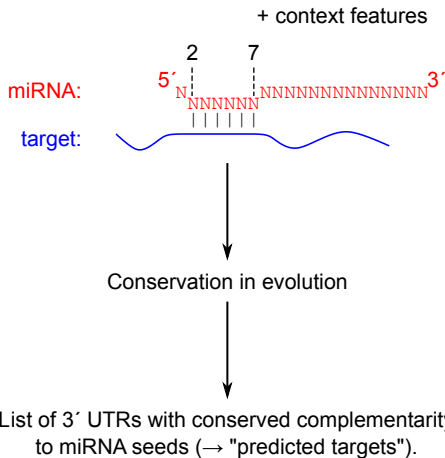
Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

miRNA target prediction



Even high-throughput identification needs to assess conservation.

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

False positives in target identification

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

False positives in target identification

Some 3' UTR seed matches are conserved for miRNA-independent reasons.

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

False positives in target identification

False positives in
miRNA targets

Some 3' UTR seed matches are conserved for
miRNA-independent reasons.

Controversy:

- ▶ [Friedman *et al.*, 2009](#): still many predicted targets after
subtracting estimated false positive counts.

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

False positives in target identification

False positives in
miRNA targets

Some 3' UTR seed matches are conserved for
miRNA-independent reasons.

miRNA target
prediction

False positives in
target
identification

Controversy:

Binding site
evolution after
miRNA loss

- ▶ [Friedman *et al.*, 2009](#): still many predicted targets after subtracting estimated false positive counts.
- ▶ [Pinzón *et al.*, 2017](#): seed match in many targets is more conserved than the miRNA itself.

Implications in
molecular oncology

Conclusion

Supplementary
data

Binding site evolution after miRNA loss

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Binding site evolution after miRNA loss

After loss of a *complete* miRNA family (*), how long do its seed matches persist ?

(*): family: all the miRNAs sharing the same seed sequence

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Binding site evolution after miRNA loss

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

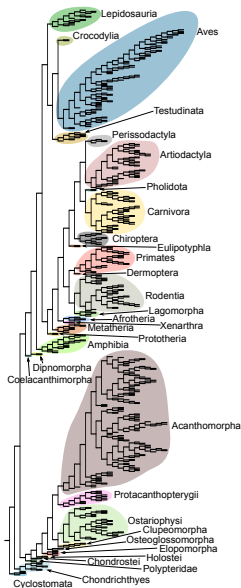
Conclusion

Supplementary
data

Binding site evolution after miRNA loss

False positives in
miRNA targets

522 vertebrate genomes:



miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Binding site evolution after miRNA loss

False positives in
miRNA targets

Clade	miRNA family
Feliformia	miR-541
Ferae	miR-598
Mammalia	miR-456
Eutheria	miR-2188
Myomorpha	miR-656
Mammalia	miR-460b
Feloidea	miR-676
Cervidae+Bovidae	miR-676
Whippomorpha	miR-769
Cervidae+Bovidae	miR-509
Protacanthopterygii	miR-31
Myomorpha	miR-885
Myomorpha	miR-3959
Theria	miR-458
Pinnipedia+Musteloidea	miR-297
Bovidae	miR-325-3p
Cervidae+Bovidae	miR-514

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Binding site evolution after miRNA loss

False positives in
miRNA targets

Clade	miRNA family
Feliformia	miR-541
Ferae	miR-598
Mammalia	miR-456
Eutheria	miR-2188
Myomorpha	miR-656
Mammalia	miR-460b
Feloidea	miR-676
Cervidae+Bovidae	miR-676
Whippomorpha	miR-769
Cervidae+Bovidae	miR-509
Protacanthopterygii	miR-31
Myomorpha	miR-885
Myomorpha	miR-3959
Theria	miR-458
Pinnipedia+Musteloidea	miR-297
Bovidae	miR-325-3p
Cervidae+Bovidae	miR-514

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

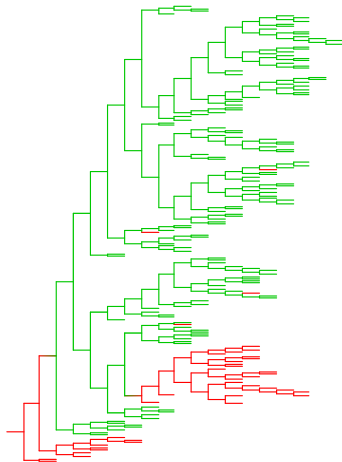
Implications in
molecular oncology

Conclusion

Supplementary
data

Binding site evolution after miRNA loss

miR-656 family:



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

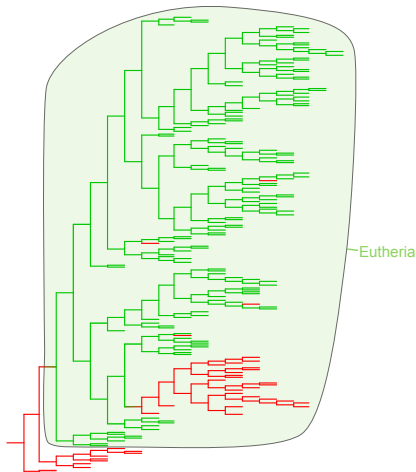
Implications in
molecular oncology

Conclusion

Supplementary
data

Binding site evolution after miRNA loss

miR-656 family:



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

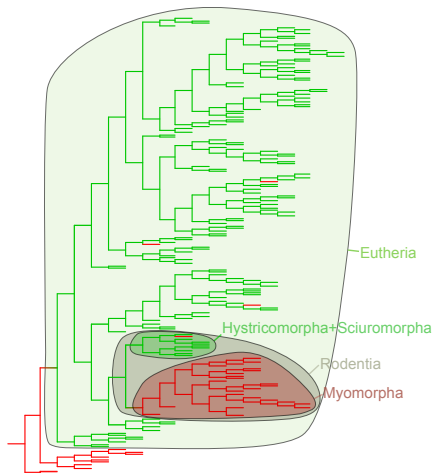
Implications in
molecular oncology

Conclusion

Supplementary
data

Binding site evolution after miRNA loss

miR-656 family:



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Binding site evolution after miRNA loss

After miR-656 family loss:

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

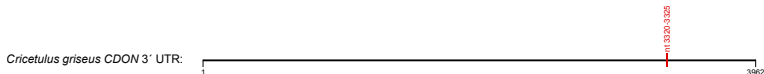
Implications in
molecular oncology

Conclusion

Supplementary
data

Binding site evolution after miRNA loss

After miR-656 family loss:



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Binding site evolution after miRNA loss

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

After miR-656 family loss:



In Rodentia:

Site	Conservation vs. rest of UTR
	<i>p</i> -value
nt 3320–3325	0.02815

Binding site evolution after miRNA loss

False positives in
miRNA targets

After miR-656 family loss:

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Cricetulus griseus CDON 3' UTR:



Acceleration of evolution in subclades:

Site	Clade of loss <i>p</i> -value
nt 3320–3325	0.03585

Binding site evolution after miRNA loss

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

After miR-656 family loss:



Acceleration of evolution in subclades:

Site	Clade of loss <i>p</i> -value	Sister clade <i>p</i> -value
nt 3320–3325	0.03585	0.50000

Binding site evolution after miRNA loss

After miR-656 family loss:

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Binding site evolution after miRNA loss

False positives in
miRNA targets

After miR-656 family loss:

miRNA target
prediction

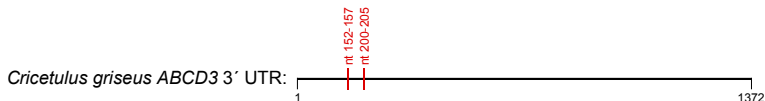
False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

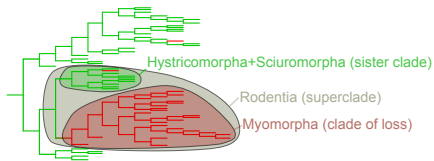
Supplementary
data



Acceleration of evolution in subclades:

Site	Clade of loss <i>p</i> -value	Sister clade <i>p</i> -value
nt 152–157	0.50000	0.00864
nt 200–205	0.23280	0.50000

Evolution of seed match count after loss



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Evolution of seed match count after loss

False positives in
miRNA targets

miRNA target
prediction

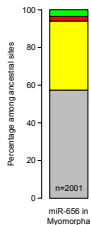
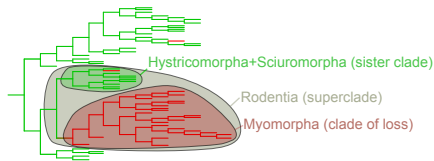
False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data



- Accelerated evolution in sister clade
- Accelerated evolution in clade of loss
- Equally conserved in clade of loss and sister clade
- Not significantly conserved in superclade

Evolution of seed match count after loss

False positives in
miRNA targets

miRNA target
prediction

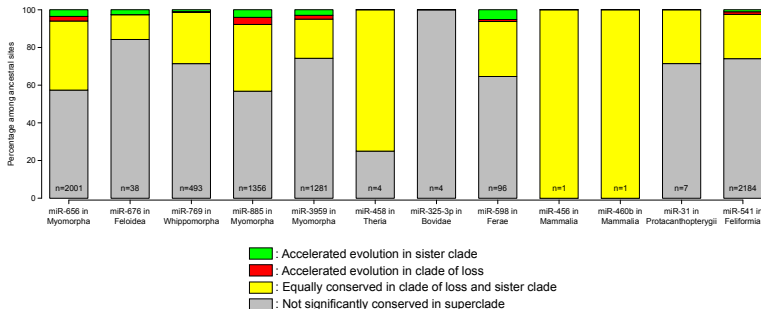
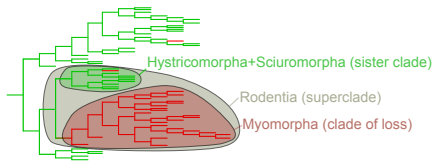
False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data



Evolution of seed match count after loss

False positives in
miRNA targets

miRNA target
prediction

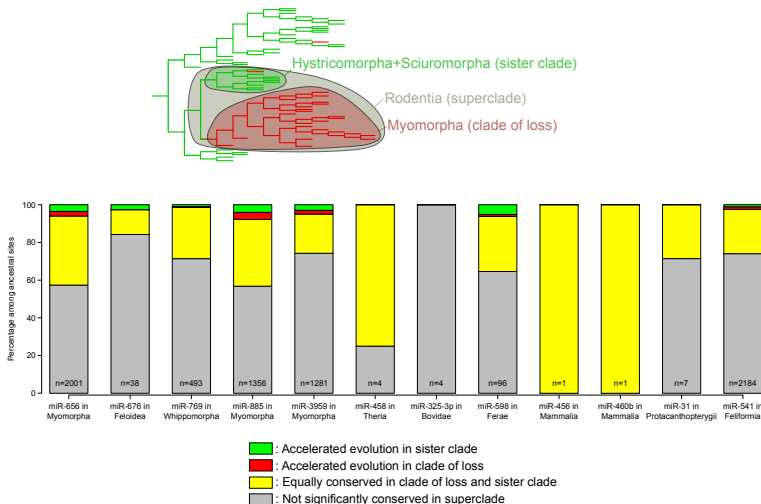
False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data



→ Most of these seed matches are conserved in a miRNA-independent manner.

Implications in molecular oncology

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

The miR-34a miRNA is a candidate tumor suppressor:

Implications in molecular oncology

The miR-34a miRNA is a candidate tumor suppressor:

- ▶ activated by p53;

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Implications in molecular oncology

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

The miR-34a miRNA is a candidate tumor suppressor:

- ▶ activated by p53;
- ▶ occasionally lost in human cancers;

Implications in molecular oncology

False positives in
miRNA targets

The miR-34a miRNA is a candidate tumor suppressor:

- ▶ activated by p53;
- ▶ occasionally lost in human cancers;
- ▶ displays anti-proliferative activity when transfected in cell lines;

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Implications in molecular oncology

False positives in
miRNA targets

The miR-34a miRNA is a candidate tumor suppressor:

- ▶ activated by p53;
- ▶ occasionally lost in human cancers;
- ▶ displays anti-proliferative activity when transfected in cell lines;
- ▶ some oncogenes among its predicted targets.

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Implications in molecular oncology

False positives in
miRNA targets

The miR-34a miRNA is a candidate tumor suppressor:

- ▶ activated by p53;
- ▶ occasionally lost in human cancers;
- ▶ displays anti-proliferative activity when transfected in cell lines;
- ▶ some oncogenes among its predicted targets.

→ Over the last 16 years: a myriad of studies; a clinical trial (“MRX34”: a synthetic miR-34a, administered to patients with solid tumors).

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Implications in molecular oncology

False positives in
miRNA targets

The miR-34a miRNA is a candidate tumor suppressor:

- ▶ activated by p53;
- ▶ occasionally lost in human cancers;
- ▶ displays anti-proliferative activity when transfected in cell lines;
- ▶ some oncogenes among its predicted targets.

—→ Over the last 16 years: a myriad of studies; a clinical trial (“MRX34”: a synthetic miR-34a, administered to patients with solid tumors).

Failure of the trial ([Beg et al., 2017](#); [Hong et al., 2020](#)).

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Implications in molecular oncology

False positives in
miRNA targets

The miR-34a miRNA is a candidate tumor suppressor:

- ▶ activated by p53;
- ▶ occasionally lost in human cancers;
- ▶ displays anti-proliferative activity when transfected in cell lines;
- ▶ some oncogenes among its predicted targets.

→ Over the last 16 years: a myriad of studies; a clinical trial (“MRX34”: a synthetic miR-34a, administered to patients with solid tumors).

Failure of the trial (Beg *et al.*, 2017; Hong *et al.*, 2020). Mixed results in mouse (encouraging results in pre-clinical assays; but the *miR-34a*^{-/-}, *miR-34b*^{-/-}, *miR-34c*^{-/-} triple KO does not exhibit abnormal p53 response: Concepcion *et al.*, 2012).

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

miR-34a is not repressed or mutated in cancers

Following analyses: published in [Mockly *et al.*, 2022](#).

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

miR-34a is not repressed or mutated in cancers

Comparison of miRNA expression in primary tumor and normal adjacent tissue ([GDC data portal](#)).

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

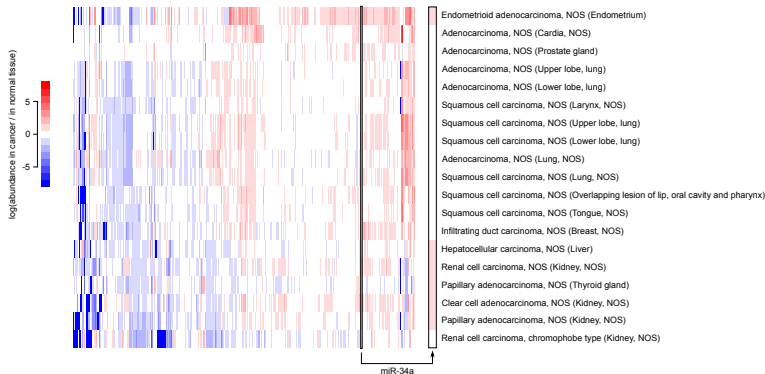
Conclusion

Supplementary
data

miR-34a is not repressed or mutated in cancers

False positives in
miRNA targets

Comparison of miRNA expression in primary tumor and
normal adjacent tissue ([GDC data portal](#)):



(cancer types with ≥ 10 analyzed cases)

► Frequency of miRNA gene mutation in cancer

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

miR-34a is not anti-proliferative in cultured cell lines

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

miR-34a is not anti-proliferative in cultured cell lines

False positives in
miRNA targets

miRNA target
prediction

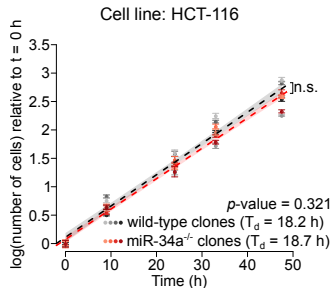
False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

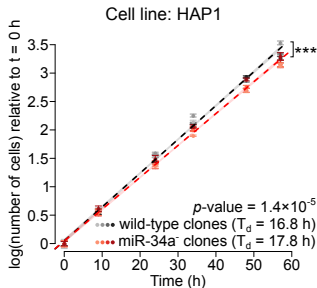
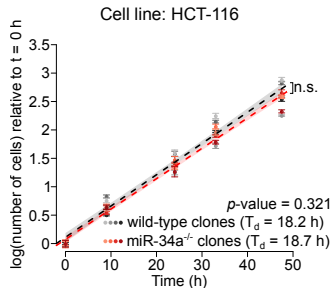
Conclusion

Supplementary
data



miR-34a is not anti-proliferative in cultured cell lines

False positives in
miRNA targets



miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

miR-34a is not anti-proliferative in cultured cell lines

False positives in
miRNA targets

—→ Proliferation arrest may be an artifact of miR-34a overexpression.

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

miR-34a is not anti-proliferative in cultured cell lines

False positives in
miRNA targets

miRNA target
prediction

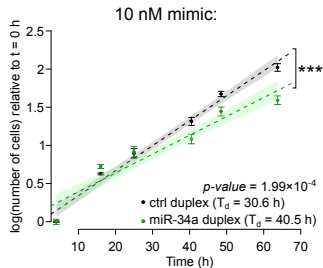
False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

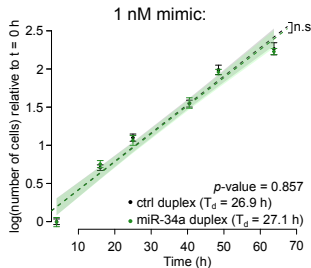
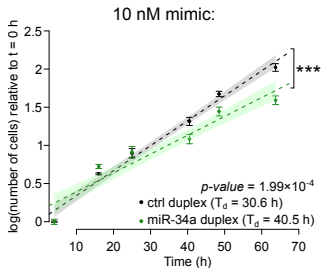
Conclusion

Supplementary
data



miR-34a is not anti-proliferative in cultured cell lines

False positives in
miRNA targets



miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

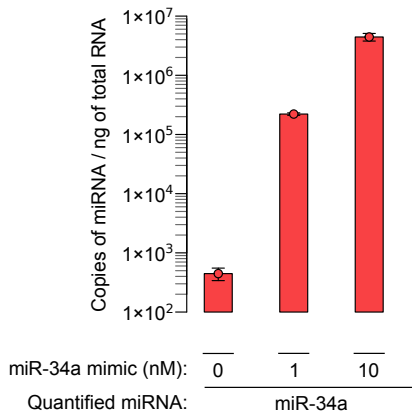
Implications in
molecular oncology

Conclusion

Supplementary
data

miR-34a is not anti-proliferative in cultured cell lines

False positives in
miRNA targets



1 nM transfection over-expresses miR-34a by >490-fold;
10 nM: by >8,000-fold.

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

miR-34a is not anti-proliferative in cultured cell lines

False positives in
miRNA targets

miRNA target
prediction

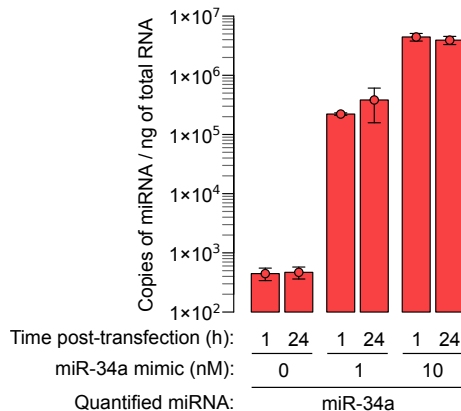
False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data



1 nM transfection over-expresses miR-34a by >490-fold;
10 nM: by >8,000-fold.

miR-34a is not anti-proliferative in cultured cell lines

False positives in
miRNA targets

miRNA target
prediction

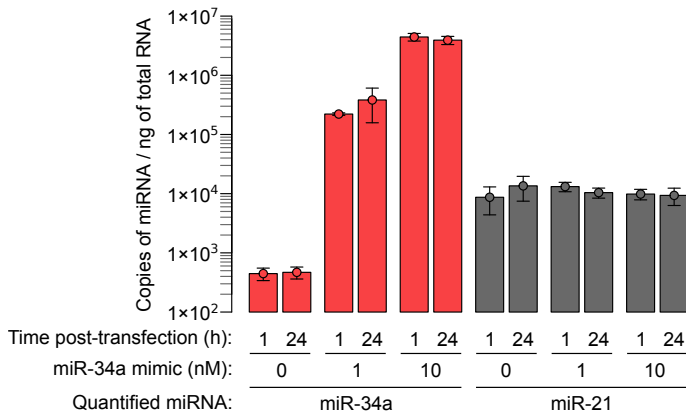
False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data



1 nM transfection over-expresses miR-34a by >490 -fold;
10 nM: by $>8,000$ -fold.

miR-34a is not anti-proliferative in cultured cell lines

False positives in
miRNA targets

miRNA target
prediction

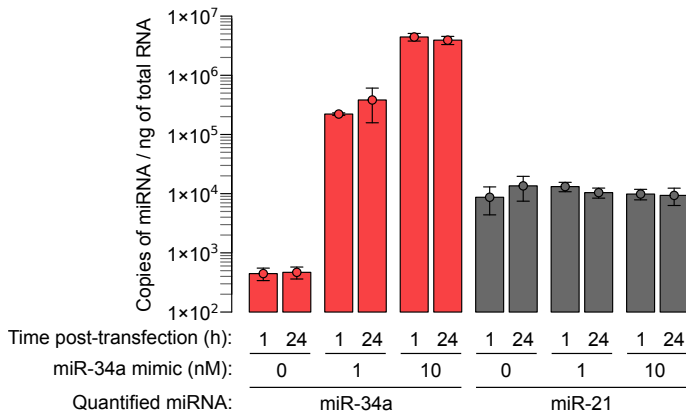
False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data



1 nM transfection over-expresses miR-34a by >490 -fold;
10 nM: by $>8,000$ -fold.

► miR-34a overexpression upon DNA damage

Conclusion

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Conclusion

High-throughput experimental target identification captures many false positives...

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Conclusion

High-throughput experimental target identification captures many false positives...

... but comparative genomics too!

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Conclusion

High-throughput experimental target identification captures many false positives...

... but comparative genomics too!

Improvement: identification of miRNA-independent conservation of binding sites.

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Conclusion

High-throughput experimental target identification captures many false positives...

... but comparative genomics too!

Improvement: identification of miRNA-independent conservation of binding sites.

On the analyzed examples: most conserved seed matches are not conserved because of the miRNA!

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Conclusion

High-throughput experimental target identification captures many false positives...

... but comparative genomics too!

Improvement: identification of miRNA-independent conservation of binding sites.

On the analyzed examples: most conserved seed matches are not conserved because of the miRNA!

→ A large part of the miRNA literature probably needs a cleanup.

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Conclusion

Erroneous miRNA target identification can have far-reaching consequences.

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Conclusion

Erroneous miRNA target identification can have far-reaching consequences.

Widely-accepted tumor suppressive function for miR-34a
—→ failed clinical trial.

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Conclusion

Erroneous miRNA target identification can have far-reaching consequences.

Widely-accepted tumor suppressive function for miR-34a
—→ failed clinical trial.

In vivo veritas: genome editing may correct a lot of false beliefs.

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

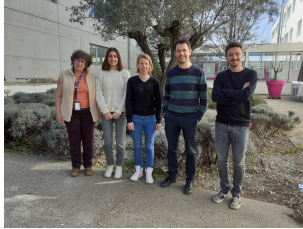
Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Acknowledgments



Current members:

Isabelle Busseau
Rémy Rodriguez

Élisabeth Houbbron
Estelle Rebecq

Former members:

Sophie Mockly
Natalia Pinzón
Laura Martinez

Germain Busto
Blaise Li
Anna Sergeeva

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Acknowledgments

False positives in
miRNA targets

miRNA target
prediction

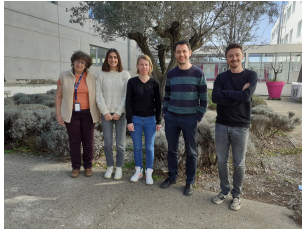
False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data



Current members:

Isabelle Busseau
Rémy Rodriguez

Élisabeth Houbron
Estelle Rebecq

Former members:

Sophie Mockly
Natalia Pinzón
Laura Martinez

Germain Busto
Blaise Li
Anna Sergeeva

Acknowledgments

False positives in
miRNA targets

miRNA target
prediction

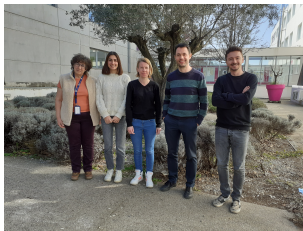
False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data



Current members:

Isabelle Busseau
Rémy Rodriguez

Élisabeth Houbron
Estelle Rebecq

Former members:

Sophie Mockly
Natalia Pinzón
Laura Martinez

Germain Busto
Blaise Li
Anna Sergeeva

Supplementary data

- ▶ Procedure for identification of miRNA family loss
- ▶ miR-656 locus alignment
- ▶ Sequence alignment of CDON seed match for miR-656
- ▶ Sequence alignment of ABCD3 seed match for miR-656
- ▶ *miR-34a* mutations in human cancers
- ▶ miR-34a over-expression upon DNA damage

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

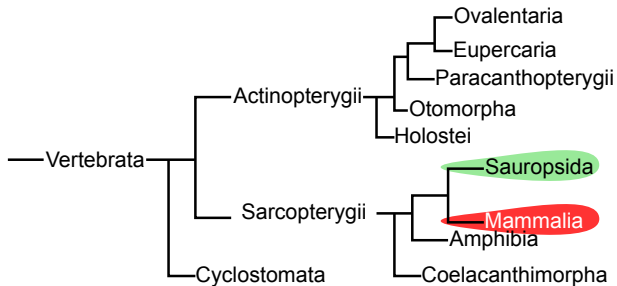
Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Strategy



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

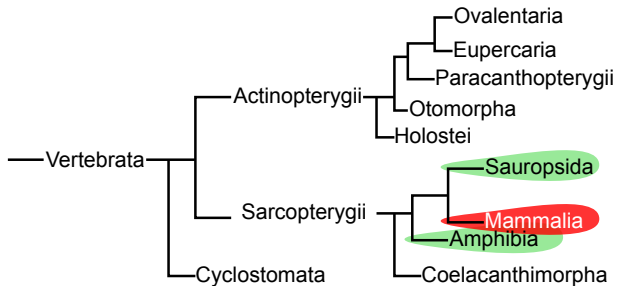
Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Strategy



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

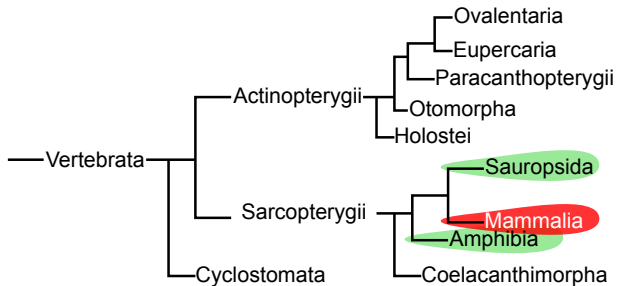
Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Strategy



More convincing if many species in clade of loss.

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

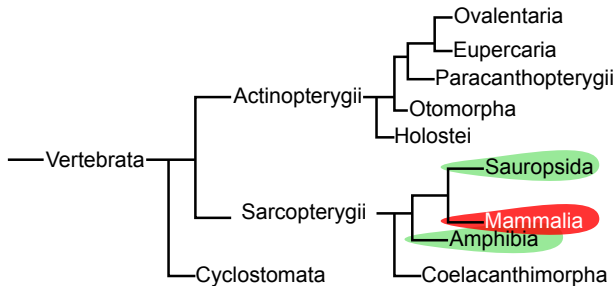
Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

Strategy



More convincing if many species in clade of loss.

≥ 10 species in clade of loss; presence in sister clade + 3 closest cousin clades.

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

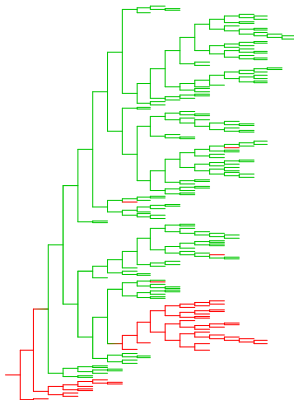
Implications in
molecular oncology

Conclusion

Supplementary
data

Strategy

miR-656 family:



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

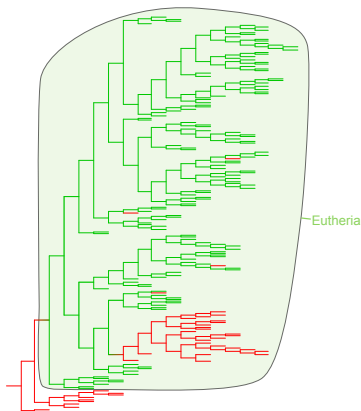
Implications in
molecular oncology

Conclusion

Supplementary
data

Strategy

miR-656 family:



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

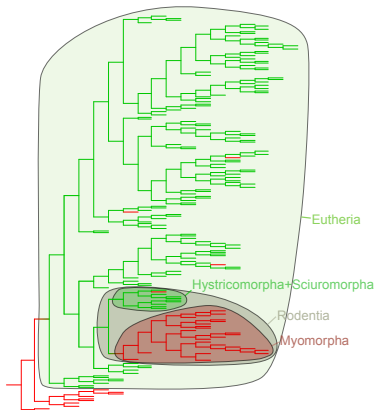
Implications in
molecular oncology

Conclusion

Supplementary
data

Strategy

miR-656 family:



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

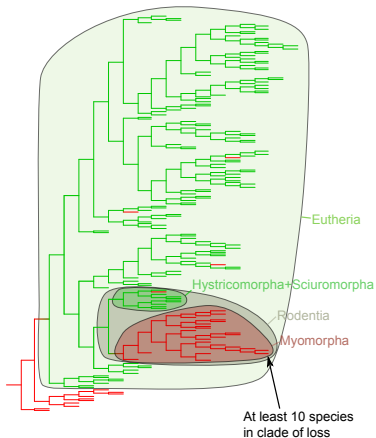
Implications in
molecular oncology

Conclusion

Supplementary
data

Strategy

miR-656 family:



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

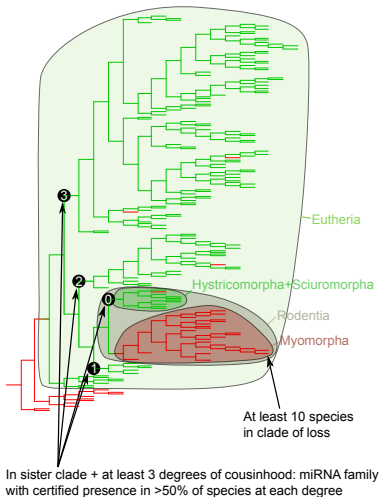
Implications in
molecular oncology

Conclusion

Supplementary
data

Strategy

miR-656 family:



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

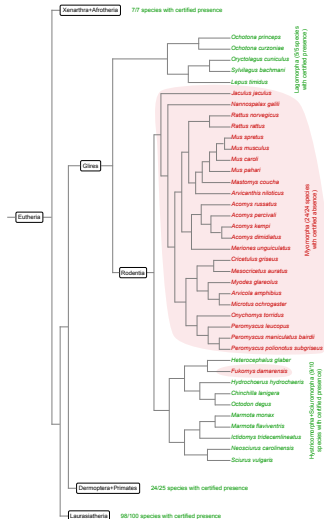
Conclusion

Supplementary
data

Observed losses

False positives in
miRNA targets

miR-656 family:



miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

False positives in miRNA targets

miR-656 family:

miRNA target prediction

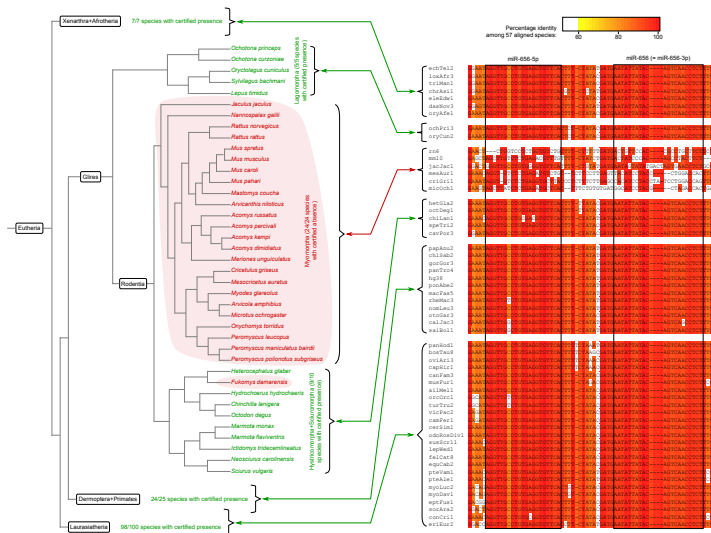
False positives in target identification

Binding site
evolution after
miRNA loss

Implications in molecular oncology

Conclusion

Supplementary data



Binding site evolution after miRNA loss

False positives in
miRNA targets

miRNA target
prediction

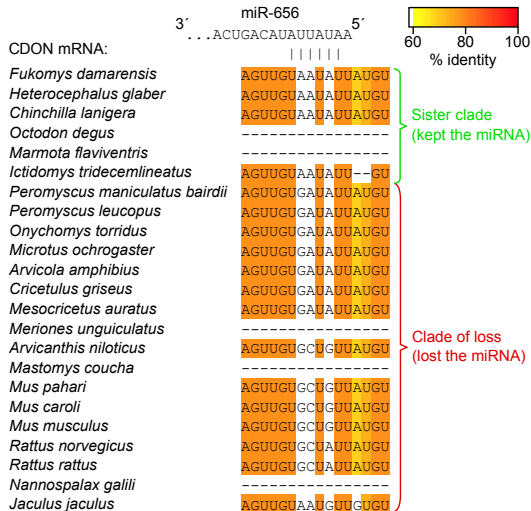
False positives in
target
identification

Binding site
evolution after
miRNA loss

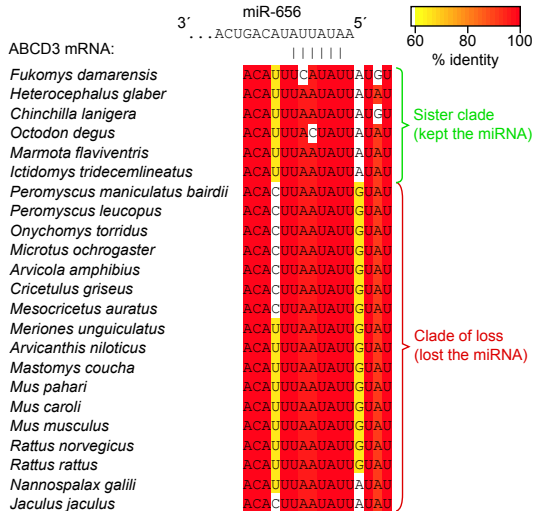
Implications in
molecular oncology

Conclusion

Supplementary
data



Binding site evolution after miRNA loss



False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

miR-34a is not repressed or mutated in cancers

False positives in
miRNA targets

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

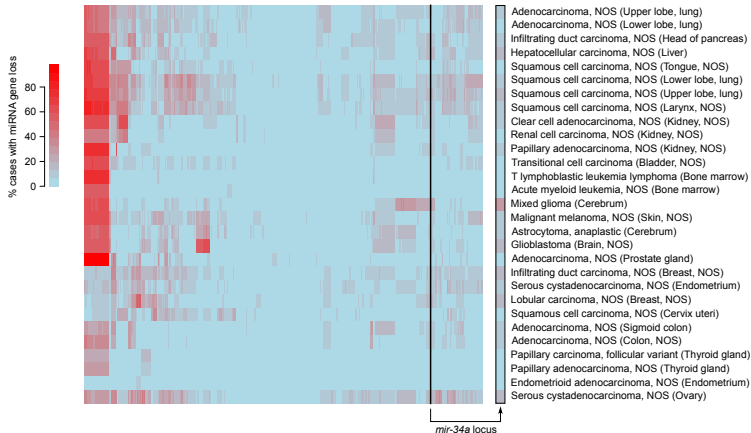
Conclusion

Supplementary
data

miR-34a is not repressed or mutated in cancers

False positives in
miRNA targets

Comparison of miRNA gene loss in cancer ([GDC data portal](#)):



(cancer types with ≥ 100 analyzed cases)

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

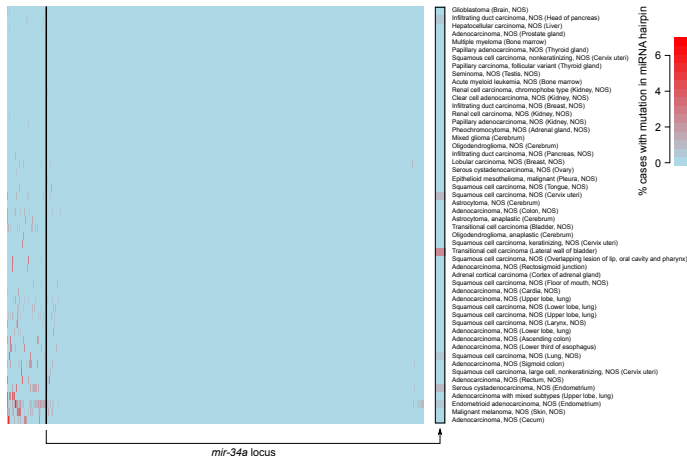
Conclusion

Supplementary
data

miR-34a is not repressed or mutated in cancers

False positives in
miRNA targets

Comparison of miRNA sequence variation in cancer (GDC data portal):



% cases with mutation in miRNA hairpin



mir-34a locus

(cancer types with ≥ 100 analyzed cases)

miRNA target
prediction

False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data

miR-34a is not anti-proliferative in cultured cell lines

False positives in
miRNA targets

miRNA target
prediction

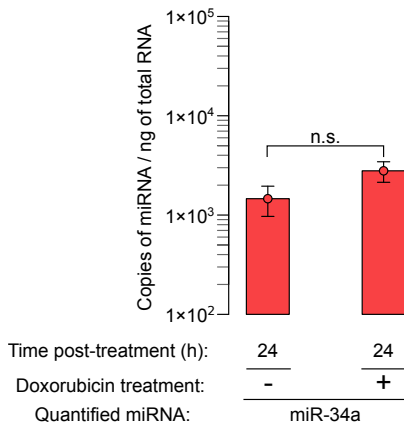
False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data



miR-34a is not anti-proliferative in cultured cell lines

False positives in
miRNA targets

miRNA target
prediction

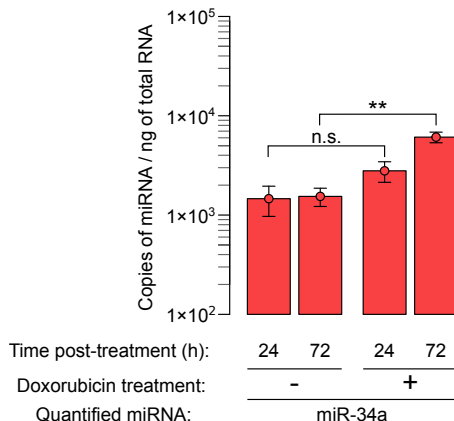
False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data



72 h treatment at IC_{50} : $4.7 \times$ increase in miR-34a.

miR-34a is not anti-proliferative in cultured cell lines

False positives in
miRNA targets

miRNA target
prediction

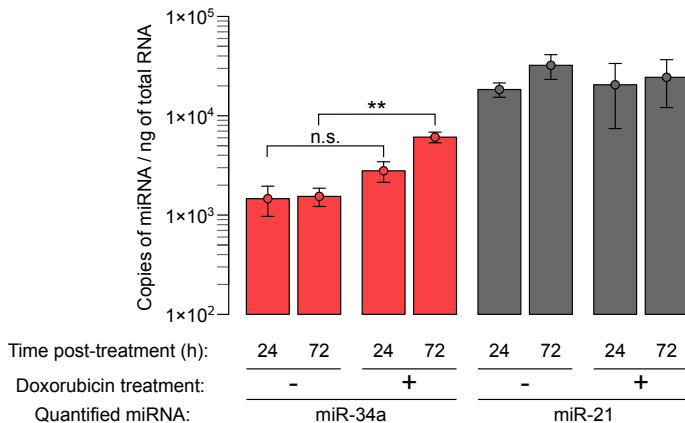
False positives in
target
identification

Binding site
evolution after
miRNA loss

Implications in
molecular oncology

Conclusion

Supplementary
data



72 h treatment at IC_{50} : $4.7 \times$ increase in miR-34a.