LINE-1 encoded reverse transcriptase (RT) in the generation of new genetic information, embryonic development and tumorigenesis

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*Rome, Italy*
Background: mouse sperm cells can internalize exogenous DNA molecules

Spadafora, BioEssays, 1998
Sperm-mediated gene transfer: molecular basis

Spadafora, BioEssays, 1998
Sperm-mediated gene transfer: summary features

1. Sperm cells can internalize exogenous DNA with which they come in contact

2. The uptake of exogenous DNA is a highly regulated process mediated by specific factors

3. The binding of exogenous DNA activates nuclear functions that are otherwise repressed in spermatozoa

4. One of these activities is an endogenous Reverse Transcriptase (RT)
Immunofluorescence detection of LINE-1 encoded RT in mouse sperm cells

Vitullo et al. 2012
Exogenous β-gal is detected in sperm cells and derived embryos

Sciananna et al., BBRC 2003
RNA-mediated Sperm-mediated “Reverse” Gene Transfer

\[ \text{β-gal expression in organs of F0 and F1} \]
\[ \text{pVLMB RNA-transformed animals} \]

Sciananna et al., BBRC 2003
EGFP copies are reverse-transcribed and spliced in sperm cells incubated with pBSKS-EGFP-int.

EGFP+int.

EGFP

Plasmid ng/10^6 sperms

<table>
<thead>
<tr>
<th>SUP</th>
<th>Nuclei</th>
<th>Plasmid DNA (ug)</th>
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<tbody>
<tr>
<td>5</td>
<td>50</td>
<td>500</td>
</tr>
<tr>
<td>50</td>
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<td>2</td>
</tr>
<tr>
<td>500</td>
<td>2</td>
<td>1</td>
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<td>50</td>
<td>2</td>
<td>0.01</td>
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Time (min.)

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<th>SUP</th>
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<tr>
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<td>15'</td>
<td>30'</td>
</tr>
<tr>
<td>5'</td>
<td>15'</td>
<td>30'</td>
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Sperm-derived reverse-transcribed EGFP is expressed in tissues of F0 mice.

Liver
Kidney
Brain

Sperm-derived reverse-transcribed EGFP is expressed in tissues of F0 mice.
Conclusions (I)

The sperm-mediated reverse gene transfer assays suggest that

- a sperm RT-mediated mechanism is responsible for the genesis of newly reverse-transcribed genetic information,

- that can be transmitted to offsprings, besides that carried by chromosomes
Detection of EGFP from tumours to germ cells: experimental outline

1. A-375 human melanoma cells stably expressing EGFP

   whole cells: RNA, DNA, proteins

   released exosomes: RNA, DNA, proteins

2. A-375/EGFP cells xenografted in athymic mice

   blood exosomes: RNA

   mature sperm cells: RNA

3. A-375/EGFP xenograft growth

Cossetti et al. Plos One 2014
Tumour marker RNAs in circulating exosomes and in germ cells of xenografted mice

A-375 melanoma cell line

<table>
<thead>
<tr>
<th>A375 RNA</th>
<th>whole cells</th>
<th>exosomes</th>
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<tbody>
<tr>
<td>EGFP infected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no RNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>non infected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>non infected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGFP infected</td>
<td></td>
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EGFP

GAPDH

Mice-derived blood exosomes

A375 cells

<table>
<thead>
<tr>
<th>EGFP infected</th>
<th>no RNA</th>
<th>non RNA</th>
<th>A-375 inoculated</th>
<th>non inoculated</th>
</tr>
</thead>
</table>

EGFP

Mice-derived sperm cells

<table>
<thead>
<tr>
<th>sperm RNA</th>
<th>no RNA</th>
<th>non inoculated</th>
<th>A-375 inoculated</th>
</tr>
</thead>
</table>

EGFP

GAPDH

Cossetti et al. PLoS ONE 2014
Human exosomes are taken up by murine spermatozoa

Interaction with non labelled exosomes
Conclusions (II)

- Human cancer cells xenografted in mice release tumor-specific RNA-containing nanovesicles (exosomes) in the circulating blood.

- RNA-mediated information flows from the soma to the germline, crossing the Weissman barrier.
Does the endogenous Reverse Transcriptase play a role in embryogenesis?
Antisense oligonucleotides targeting active LINE-1/L1 arrest early embryo development

Beraldi et al Mol Repr Dev 2006
BrdU incorporation in early mouse embryos

Vitullo et al. Mol Repr Dev 2012
Nevirapine (nonnucleoside RT Inhibitor) abolishes aphidicicolin-resistant BrdU incorporation

Vitullo et al. Mol Repr Dev 2012
LINE-1 copy number is amplified in preimplantation embryogenesis

Vitullo et al. Mol Repr Dev 2012
Conclusions (III)

- RT inhibition causes a drastic arrest of embryo development (2-4 cell stages)

- Reverse transcription takes place in both male and female zygotic pronuclei soon after fertilization

- LINE-1s are amplified throughout preimplantation development

RT activity is strictly necessary for preimplantation development
Does the endogenous Reverse Transcriptase play a role in cell proliferation and tumor growth?
Targeting human active LINE-1 retroelements by RNA interference

Oricchio et al., Oncoegne 2007
LINE1-TARGETED RNAi REDUCES CELL PROLIFERATION AND STIMULATES DIFFERENTIATION

A375 MELANOMA CELL CULTURES

PROLIFERATION

MORPHOLOGICAL DIFFERENTIATION

Oricchio et al., Oncoegne 2007
A375 cells interfered for LINE-1 exhibit reduced tumorigenicity in vivo

Oricchio et al., Oncoegne 2007
Efavirenz inhibits proliferation in human transformed cell lines

Efavirenz inhibits proliferation in human transformed cell lines. Efavirenz in inhibits proliferation in human transformed cell lines. Sciamanna et al. Oncotarget 2013
RT inhibitors induce morphological differentiation of melanoma cells

A-375 melanoma cells exposed to RT inhibitors acquire:
- dendritic-like extensions
- flattened shape
- high adhesion

These features are typical of melanoma cells induced to differentiate
In vivo anti-tumor effectiveness of RT inhibitors
Human tumor cell lines xenografted in nude mice:

- PC3 prostate carcinoma
- HT29 colon carcinoma
- A375 melanoma
- H69 small cell lung carcinoma

Treatment with RT inhibitors started one day, or one week, after tumor xenograft
Efavirenz inhibits the growth of H69 small cell lung carcinoma in nude mice

Untreated

Efavirenz-treated

25 days

40 days
RT inhibitors reduce the growth of tumor xenografts *in vivo*
Sciamanna et al., Oncogene 2005

**H69** small lung carcinoma

**A375** melanoma

**HT29** colon carcinoma

**PC3** prostate carcinoma

- ctrl
- Efavirenz starting 1 day after tumor cell inoculation
- Efavirenz starting 1 week after tumor cell inoculation
- Efavirenz interrupted after 14 days
TESTING OF RT INHIBITORS: SUMMARY

The results with animal models suggest that RT can be regarded as a target in a novel cancer differentiation therapy.

A phase II trial with the RT inhibitor Efavirenz on patients with bone metastasis of primary prostate carcinoma is ongoing (Institut Bergoniè, Bordeaux, France)

A “junk DNA”-based anticancer therapy?
RT expression and activity during breast cancer progression

**LINE-1 RT**

**Merge with DNA**

**LINE-1 RT protein**

**RT activity**

Gualtieri et al. Oncotarget 2013
Enhanced transcription and copy number amplification of LINE-1 and SINE B1 during breast cancer progression
<table>
<thead>
<tr>
<th>Name</th>
<th>EFV modulation</th>
<th>Modulation in cancer</th>
<th>Biological function</th>
<th>Cancer type</th>
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<tbody>
<tr>
<td>miR-21</td>
<td>down</td>
<td>up</td>
<td>Correlated with invasion and metastasis</td>
<td>lung, colorectal</td>
</tr>
<tr>
<td>miR-33a</td>
<td>down</td>
<td>up</td>
<td>Dysregulated in bone metastasis from primary prostate cancer</td>
<td>prostate</td>
</tr>
<tr>
<td>miR-181a</td>
<td>down</td>
<td>up</td>
<td>Related with shortened disease-free survival, highly upregulated in osteosarcoma</td>
<td>osteosarcoma</td>
</tr>
<tr>
<td>miR-199b</td>
<td>down</td>
<td>up</td>
<td>Dysregulated in metastasis</td>
<td>brain</td>
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<tr>
<td>miR-34b</td>
<td>up</td>
<td>down</td>
<td>Downregulated in metastasis, reactivated upon drug treatment inhibits tumor growth and lymph node metastasis</td>
<td>colorectal, melanoma, head and neck</td>
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<tr>
<td>miR-125b</td>
<td>up</td>
<td>down/up</td>
<td>Downregulated in breast and upregulated in i cancer, association with cancer metastasis</td>
<td>breast, colorectal</td>
</tr>
<tr>
<td>miR-146a</td>
<td>up</td>
<td>down</td>
<td>Inversely correlated expression with cancer progression and metastasis</td>
<td>prostate, breast</td>
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<tr>
<td>miR-148a</td>
<td>up</td>
<td>down</td>
<td>Downregulated in metastasis, acts as metastasis suppressor inhibiting tumor growth and lymph node metastasis</td>
<td>colorectal, melanoma, head and neck</td>
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<tr>
<td>miR-193b</td>
<td>up</td>
<td>down</td>
<td>Inversely correlated expression with cancer progression, invasion and metastasis</td>
<td>breast</td>
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<tr>
<td>miR-204</td>
<td>up</td>
<td>down</td>
<td>Highly reduced expression in cancer progression; overexpression suppresses invasiveness and acts as metastasis suppressor</td>
<td>head and neck</td>
</tr>
</tbody>
</table>
RT control of the cancer cell transcriptome: a model

Sciamanna et al. Oncotarget 2013
Identification or RNA:DNA hybrid structures in cancer cells through CsCl density gradient centrifugation (Sciamanna et al. 2013)

Linear DNA  
Circular DNA  
Hybrid RNA:DNA

A375  
PC3  
WI38  
A375 EFV

8 17 21 25 CN

DNAse I  
RNAse /DNAse I

8 21

- - +  - - +

Alu  
LINE-1 ORF2

DNAse I  
RNAse /DNAse I

- - +  - - +  - + -

- - +  - + -  - - +

100 500 850

850 650 400 500 100

1402 1400 1398 1396 1394 1392 1390 1388 1386 1384 1382 1380 1378 1376 gr/ml

fraction
Conclusions (IV)

- LINE-1 and Alu elements are up-regulated, both in expression and in copy number, during tumor progression.

- LINE-1-encoded ORF2 protein, hence RT, increases during tumor progression.

- RT inhibition reduces cancer cell proliferation and promotes differentiation; also antagonizes cancer progression in animal models in vivo.

- RT inhibition globally reprogrammes the expression profile in cancer cells.

- An RT-dependent cancer-promoting mechanism plays a causative role in cancer onset and progression.
A. Inhibition of endogenous RT in early embryos

- Zygote
- Normal development
- Arrest of development (irreversible)
- Blastocyst

B. Inhibition of endogenous RT in transformed cells

- Transformed cell
- Dedifferentiation
- Tumor progression
- Differentiated cell
- RT inhibitor removal
- Differentiated cell (reversible)
- Transformed cell
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