XIST promoter methylation status as putative molecular biomarker for testicular germ cell tumors

João Lobo

Sandra Nunes, Vera Gonçalves, Daniela Barros-Silva, Annette van der Berg, Ad Gillis, Leendert HJ Looijenga, Carmen Jerónimo, Rui Henrique

No conflicts of interest
“Germ cell tumors are at the crossroads between developmental biology and cancer”

"Developmental biology as a driver for uncovering novel disease biomarkers"
**XIST** = *X*-inactive specific transcript (IncRNA, Xq13.2)

X-inactivation centers

Transcription of *XIST* RNA from the *XIST* gene

*XIST* RNA induces chromosome-wide coating and silencing

X-chromosome inactivation

Lobo *et al.* (submitted)
**XIST** = X-inactive specific transcript (IncRNA, Xq13.2)

- **Xa** and **Y** chromosomes in males (XY).
- **Xa**, **Xi**, and **Y** chromosomes in females (XX).
- **Xa**, **Xi**, **Xi**, and **Y** chromosomes in testicular germ cell tumors (XY with supernumerary X).

**Male (XY):**
- NOT EXPRESSED
- METHYLATED

**Female (XX):**
- EXPRESSED
- DEMETHYLATED

**Testicular germ cell tumors:**
- EXPRESSED
- DEMETHYLATED

Lobo et al. (submitted)
XIST promoter (++region IV) consistently demethylated in TGCTs, (++Seminomas)

No demethylated fragments in somatic male cancers
Infertility with Testicular Cancer

Kevin A. Ostrowski, MD, Thomas J. Walsh, MD

Hypogonadism and Sexual Dysfunction in Testicular Tumor Survivors: A Systematic Review

Sandro La Vignera, Rossella Cannarella, Ylenia Duca, Federica Barbagallo, Giovanni Burgio, Michele Compagnone, Andrea Di Cataldo, Aldo E. Calogero and Rosita A. Condorelli

Young-adults

Overall good prognosis

Long-term treatment side effects

Costa and Lobo et al. Epigenomics 2017
<table>
<thead>
<tr>
<th>Johnsen’s Score</th>
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<th>Testicular Pathology</th>
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JOHNSEN’S SCORE

Laborious
Subjective
(low reproducibility)

Invasive (biopsy)
**Rationale**

*XIST is expressed (=demethylated) in the testis (spermatogenesis) around the time of entering meiosis*

*This process of XIST activation in spermatogenesis is regulated by METHYLATION*

Richler *et al.* Nat Genet 1992
To explore the role of (de)methylated \textit{XIST} promoter as a candidate biomarker

- For TGCTs
- For spermatogenesis status

Samples (2005-2017)

Bisulfite treatment (Zymo kit)

DNA extraction (Norgen kit/PC8)

qMSP (ABI 7500 RT PCR System)

250 TGCTs (tumor components)

54 testicular parenchyma (JS)

4 (T)GCT cell lines

Same primer set reported by Kawakami \textit{et al}
Validation of the series

Testicular germ cell tumors: revisiting a series in light of the new WHO classification and AJCC staging systems, focusing on challenges for pathologists

João Lobo MD<sup>a</sup>, Ana Laura Costa MSc<sup>b</sup>, Bárbara Vilela-Salgueiro MSc<sup>b</sup>, Ângelo Rodrigues MD<sup>a</sup>, Rita Guimarães BSc<sup>a</sup>, Mariana Cantante BSc<sup>a</sup>, Paula Lopes BSc<sup>a</sup>, Luís Antunes MSc<sup>d</sup>, Carmen Jerónimo PhD<sup>b</sup>, Rui Henrique MD, PhD<sup>a</sup>, PhD<sup>b</sup>, PhD<sup>c</sup>
**XIST methylated fragment – TGCTs**

**Methylated XIST promoter fragment**

- **Testicular parenchyma** vs. **TGCT samples**
- **Testicular parenchyma** vs. **SE samples**
- **SE samples** vs. **NS samples**
- **SE** vs. **EC** vs. **YST** vs. **CH** vs. **TE**
Demethylated XIST promoter fragment

Testicular parenchyma  TGCT samples
Demethylated XIST promoter fragment

A

B

**XIST demethylated fragment – TGCTs**
Demethylated XIST promoter fragment

(T)GCT cell lines

Percent of XIST demethylation (%)

TCam-2  NT2  NCCIT  2102Ep

High Resolution Melting (HRM) analyses
### XIST demethylated fragment - spermatogenesis

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**Graph:**
- Demethylated XIST fragment (relative levels) vs. Johnsen Score
- $r_s = 0.75$ (p<0.0001)
XIST demethylated fragment - spermatogenesis

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**Context**

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To explore the role of (de)methylated \textit{XIST} promoter as a candidate biomarker

✓ For TGCTs

✓ For spermatogenesis status

\textbf{Testicular germ cell tumors}

\textbf{Early (differential) diagnosis}

\textbf{Follow-up}

\textbf{Especially for Seminoma}

\textbf{Spermatogenesis status}

\textbf{Improve selection of patients for TESE or other fertility preservation techniques
Detection of TGCTs in liquid biopsies (serum/plasma)
Patient monitoring

Upfront assessment of male patients' fertility (semen samples)

HRM – High-resolution melting
Supervising team:
Rui Henrique
Carmen Jerónimo
Leendert Looijenga