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
Epigenetics

Dev. Biology

BIOMARKERS

Early diagnosis
Resistance
Prognosis
Fertility preservation
Response to therapy
Targeted treatment

Lobo et al. Hum Pathol 2018
“Germ cell tumors are at the **crossroads** between developmental biology and cancer”

“Developmental biology as a driver for uncovering novel disease biomarkers”

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

X-chromosome inactivation
**XIST** = X-inactive specific transcript (IncRNA, Xq13.2)

- **Xa** = inactive X chromosome in male (XY) individuals.
- **Xi** = inactive X chromosome in female (XX) individuals.
- **Y** = Y chromosome.

**Methylation states:**
- **M** = methylated
- **W** = demethylated

**Expression states:**
- **NOT EXPRESSED**
- **EXPRESSED**

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

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

**Testicular germ cell tumors (XY with supernumerary X):**
- EXPRESSED
- DEMETHYLATED

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Lobo et al. (submitted)
Rationale

XIST promoter (++region IV) consistently demethylated in TGCTs, (++Seminomas)

No demethylated fragments in somatic male cancers


Kawakami et al. Lancet 2004
JOHNSEN’S SCORE

Laborious

Subjective
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 XIST promoter as a candidate biomarker for TGCTs and spermatogenesis status.

Samples (2005-2017):
- 250 TGCTs (tumor components)
- 54 testicular parenchyma (JS)
- 4 (T)GCT cell lines

Bisulfite treatment (Zymo kit)

DNA extraction (Norgen kit/PC8)

qMSP (ABI 7500 RT PCR System)

Same primer set reported by Kawakami et al.
XIST methylated fragment – TGCTs

Methylated XIST promoter fragment

Testicular parenchyma TGCT samples

Methylated XIST fragment (relative levels)

Testicular parenchyma SE samples

Methylated XIST fragment (relative levels)

SE samples NS samples

Methylated XIST fragment (relative levels)

SE EC YST CH TE

Methylated XIST fragment (relative levels)
Demethylated XIST promoter fragment

![Graph showing demethylated XIST fragment levels in testicular parenchyma and TGCT samples.](image)
**XIST demethylated fragment – TGCTs**

Demethylated *XIST* promoter fragment

[A] and [B] show the relative levels of demethylated XIST fragment across different conditions. The graphs compare the levels between SE and NS in [A], and SE, EC, YST, CH, and TE in [B]. Significant differences are indicated by asterisks: ***p < 0.001, *p < 0.05.
Demethylated XIST promoter fragment

(T)GCT cell lines

Percent of XIST demethylation (%)

TCam-2  NT2  NCCIT  2102Ep

High Resolution Melting (HRM) analyses
<table>
<thead>
<tr>
<th>Johnsen's Score</th>
<th>Description</th>
<th>Testicular Pathology</th>
<th>Cell Type Instruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fibrosis</td>
<td></td>
<td>Leydig Cell and Peritubular Myoid Cell</td>
</tr>
<tr>
<td>2</td>
<td>Sertoli only</td>
<td></td>
<td>Sertoli Cell</td>
</tr>
<tr>
<td>3</td>
<td>Spermatogonia only</td>
<td></td>
<td>Spermatogonia</td>
</tr>
<tr>
<td>4</td>
<td>Few spermatocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Many spermatocytes</td>
<td></td>
<td>Spermatocyte</td>
</tr>
<tr>
<td>6</td>
<td>Few early spermatids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>No spermatzoa, no late spermatids, many early</td>
<td>Round Spermatid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>spermatids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Less than five spermatzoa, few late spermatids</td>
<td>Elongated Spermatid</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Slightly impaired, many late spermatids,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>disorganized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Normal spermatogenesis</td>
<td></td>
<td>Spermatozoon</td>
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**Graph:**

The graph shows the correlation between the Demethylated XIST fragment (relative levels) and the Johnsen Score. The correlation coefficient $r_s = 0.75$ is statistically significant ($p < 0.0001$).

**Legend:**

- $r_s$ = Spearman's rank correlation coefficient
- $p$ = Probability value
**XIST demethylated fragment - spermatogenesis**

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<th>Context</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<td>JS≥4 vs JS&lt;4</td>
<td>75.7</td>
<td>100</td>
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AUC: 0.87
95% CI: 0.77-0.97
p<0.0001
To explore the role of (de)methylated \textit{XIST} promoter as a candidate biomarker

- For TGCTs
- For spermatogenesis status
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Thank you for your attention!