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# Loss of the Y Chromosome in Esophageal Adenocarcinoma - Epiphenomenon or Oncogenic Event?

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## ABSTRACT

**The prevalence of Barrett's esophagus, caused by chronic exposure of the lower esophagus to gastric acid, is a major risk factor for developing esophageal adenocarcinoma. In the last decades, the incidence of esophageal adenocarcinoma has increased significantly in Western countries in both, men and women. However, men are diagnosed with Barrett's mucosa twice as often, and, interestingly, will develop esophageal adenocarcinoma up to nine times more likely than women. The reasons for this gender-specific susceptibility to Barrett's-associated neoplasia are currently unknown. Loss of the Y chromosome (LOY) is commonly observed in aging men and associated with a wide spectrum of diseases such as cancers, including esophageal adenocarcinoma. However, the phenotypic consequences of LOY remain elusive and, to our knowledge, have not been directly investigated to date. Here, we test the functional, genetic and genomic consequences of LOY by deleting the entire Y chromosome in Barrett's cell lines via CRISPR-Cas9 directed fragmentation of the Y centromere. Our study will help clarifying, whether the actual LOY directly contributes to tumorigenesis and the male predominance of esophageal adenocarcinoma.**

## PROJECT OUTLINE

**Cell lines:**

1. **Normal Mucosa:**
EPC-1 (XY)
EPC-2 (XY)

2. **Barrett's Mucosa:**
CP-A (metaplasia) (XY)
CP-B (dysplasia) (XY)

3. **EAC:**
Eso-26 (XY)

→

**CRISPR-Cas9 directed fragmentation of the Y centromere:**

sgRNAs targeted against the satellite array of the Y centromere (DYZ3): Each sgRNA targets the Y centromere 52 times

→

**Confirmation of Y chromosome Deletion:**

1. PCR
2. FISH
3. SNP-Array

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**Test for Genetic and Genomic Effects:**
- 3'prime Seq.
- WGS
- bisulfite Seq.
- ATAC Seq.

**Test for Functional Effects:**
- proliferation/cell cycle
- invasion
- migration/wound-scratch

## PROOF OF CONCEPT

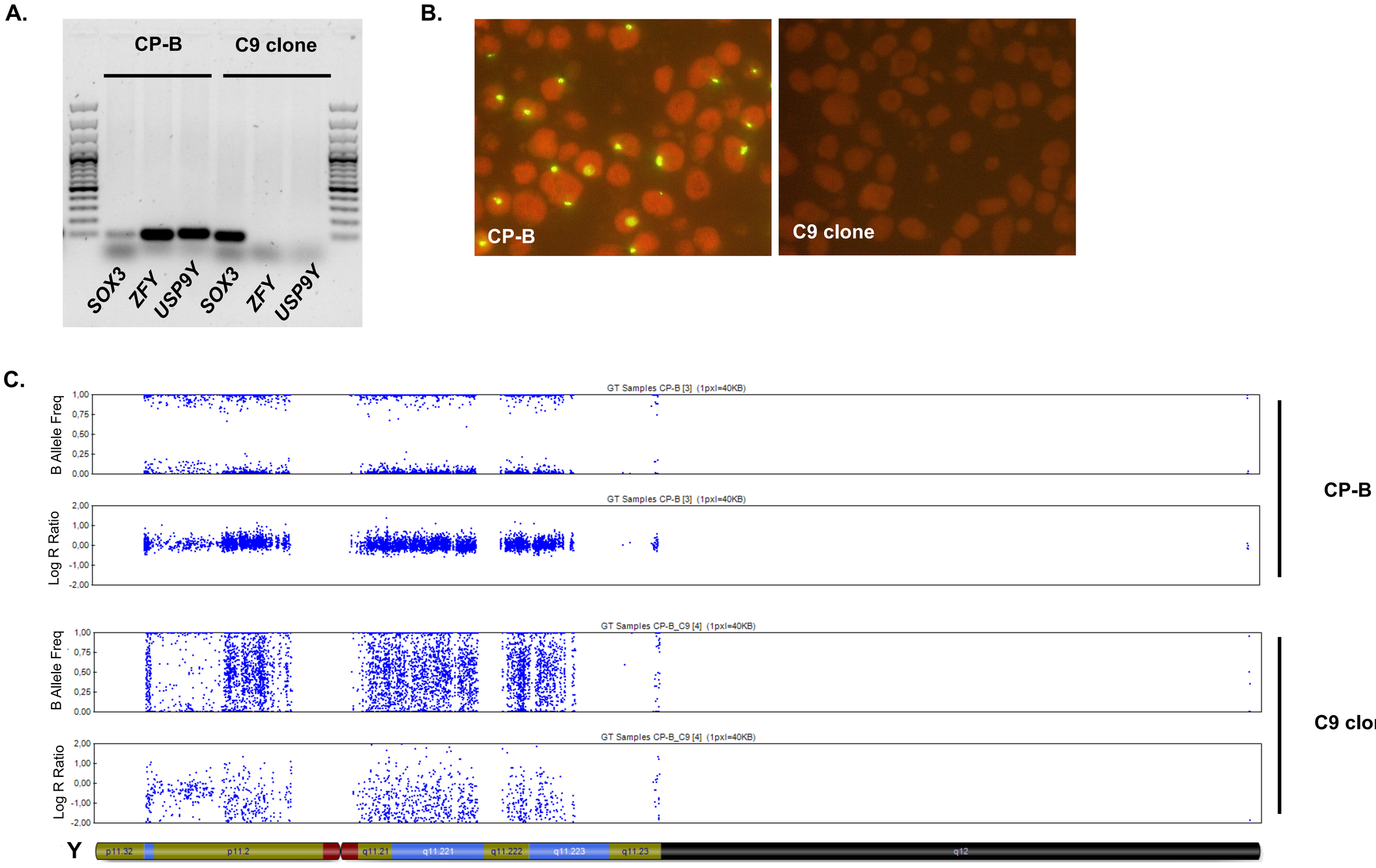


**Fig 1 CRISPR-Cas9 directed deletion of the entire Y chromosome in a Barrett's mucosa cell line (CP-B). (A)** Genomic PCR to confirm CRISPR-Cas9 directed deletion of the Y chromosome: *SOX3* (gene located on the X chromosome) was amplified in DNA extracted from CP-B cells and in DNA extracted from the C9 clone. *ZFY* (gene located on the short arm of the Y chromosome) and *USP9Y* (gene located on the long arm of the Y chromosome) was only amplified in DNA extracted from CP-B cells but not in DNA extracted from the C9 clone . **(B)** FISH to confirm CRISPR-Cas9 directed deletion of the Y chromosome: representative Y chromosome signals of the short (red signal) and long (green signal) arm in CP-B cells (left side image) and no signals in the C9 clone (right side image) (magnification x630). **(C)** SNP-Array to confirm CRISPR-Cas9 directed deletion of the Y chromosome.

## Links to relevant publications

https://doi.org/10.3322/caac.21185 https://doi.org/10.3390/cancers12071743 https://doi.org/10.1016/j.ymthe.2017.05.021 https://doi.org/10.1186/s13059-017-1354-4