Loss of Y chromosome in male cancer cells

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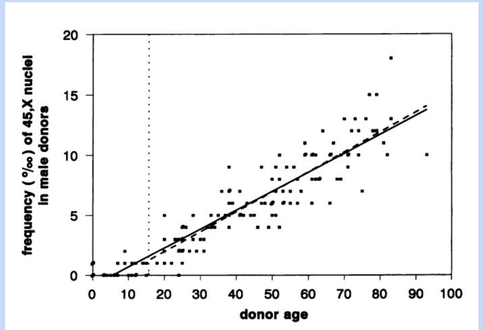
Abstract

Higher mortality rates among males with cancer have been studied for many decades, but little research on how and why this is true exists. Studies have shown that in the cells of male cancer patients and even healthy aging males, part or all of the Y chromosome is missing. Evidence of Loss of Y (LOY) paired with evidence of higher mortality rates among male cancer patients raises the question of how the absence of Y might affect cancer progression and which genes may be responsible. This project seeks to answer these questions by detecting the presence or absence of Y in cancer cell lines and reintroducing specific genes into these cell lines to investigate their effects on cancer progression. This project makes use of techniques including cloning and Fluorescence In Situ Hybridization (FISH).

Background

Chromosome Loss with Age

- The frequency of LOY has been found to increase with age.



- **Figure 1.**[1] Graph showing reported increasing frequency of LOY in male donors

Chromosome Loss with Disease

- In 1985, a study showed that out of 400 male patients with a form of leukemia, 13 were missing part or all of their Y chromosome.

Table 2 Treatment and outcome of patients with AML and − Y chromosome

Patient no.	Treatment and on CR	Bone marrow cytogenetics on CR	Remission duration (mo)	Survival (mo)
1	AD-OAP (2)	3/76 46,XY (6 cells)	9	12
2	AD-OAP	—	—	1 wk (sepsis)
3	Lithium carbonate	—	—	21
4	AD-OAP	—	—	1 (cardiac arrest)
5	AD-OAP (2)	10/76 100% 46,XY (15 cells)	18 (first CR) 9 (second CR)	31
6	AD-OAP (1)	3/78 100% 46,XY (20 cells) (not banded)	10	16
7	ROAP	—	—	1 (sepsis)
8	AD-OAP (3)	5/80 100% 46,XY (23 cells)	24	29
9	Lithium carbonate	—	—	7
10	AD-OAP (1)	11/81 100% 46,XY (16 cells) (not banded)	—	1 (Died of acute myocardial infarction 11/81)
11	AD-OAP (1)	7/82 100% 46,XY (25 cells) 6/83 (off treatment) 100% 46,XY (25 cells)	21 +	22 +
12	AMSA-OAP (2)	4/83 100% 46,XY (23 cells)	6	14
13	AMSA-OAP	—	—	11

- **Figure 2.**[2] Table showing the treatment and outcome of 13 male patients with leukemia and LOY

  - Patients who achieved remission showed restored diploid karyotype after remission bone marrow chromosome analysis.

Methods

Detecting the Y Chromosome in Cancer Cell Lines Using FISH

- Six cell lines were chosen for probing:
  - Fibroblasts: human foreskin cells (positive control)
  - A375: female melanoma cell line (negative control)
  - Sk-Mel-2: male melanoma cell line
  - Sk-Mel-24: male melanoma cell line
  - PANC-1: male pancreatic cancer cell line
  - Mia-Paca-2: male pancreatic cancer cell line

- Cells from each cell line were plated in a 6-well plate on slips and fixed with methanol and glacial acetic acid.

- XY probe solution was applied directly to the fixed cells and denatured.

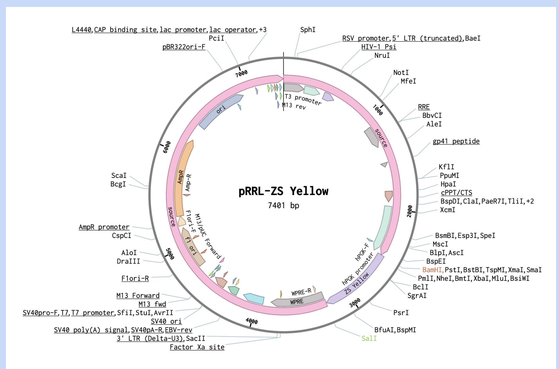
- Following overnight incubation, the cells were washed then imaged using a fluorescent microscope.

Cloning Y Genes

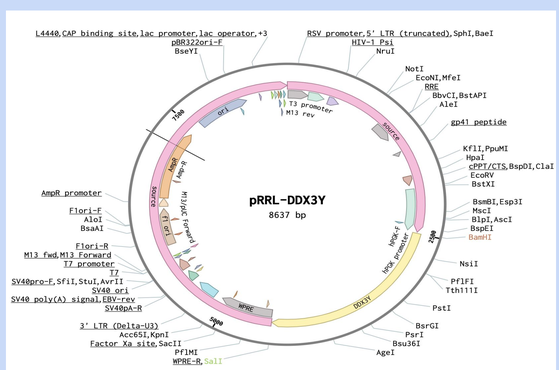
- Three Y chromosome genes were chosen for cloning based on their ubiquitous expression and high protein expression in skin.
  - DDX3Y: involved in translation and RNA metabolism
  - RPS4Y: involved in translation
  - EIF1AY: involved in translation initiation

- A pRRL vector was used to clone the Y genes; its insert (ZS-Yellow) was taken out using restriction enzymes at the BamH1 and SalI sites.

- The sequences for the genes were cut using the same restriction enzymes, ligated into the pRRL vector, and grown on plates.

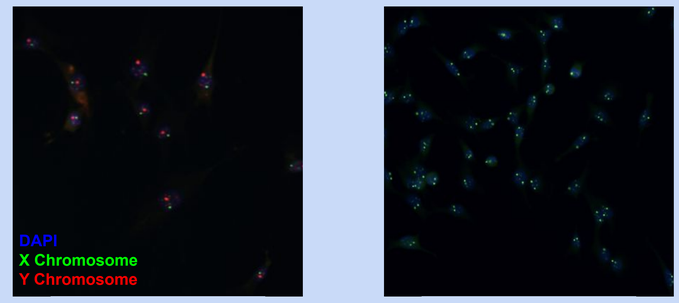


- **Figure 3.** pRRL expression vector with ZS-Yellow insert



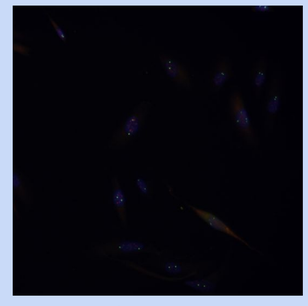
- **Figure 4.** Example of gene insertion into pRRL expression vector

Results & Data

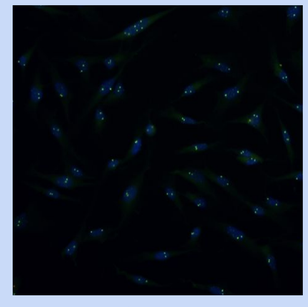


Fibroblasts

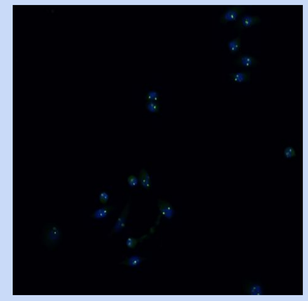
A375



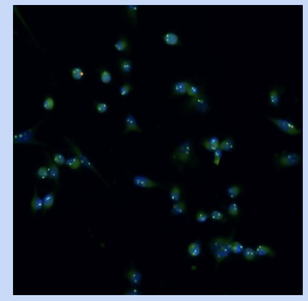
Sk-Mel-2



Sk-Mel-24



PANC-1



Mia-Paca-2

- All four male cancer cell lines tested lacked a Y chromosome.
- These results support previous findings that male cancer cells lose their Y chromosomes.

Discussion & Future Direction

More Than a Sex Determining Chromosome

- The correlation between LOY and cancer suggests that the Y chromosome is responsible for more than just sex determination; some genes may act as tumor-suppresors.
- LOY is a distinct marker for cancer in males.

Future Direction

- Reintroduce specific Y genes into cancer cell lines
- Studying LOY at different stages of cancer
- Detecting the presence of Y in male nevi and melanoma biopsies

Acknowledgements & References

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1. Guttenbach M, Koschorz B, Bernthaler U, Grimm T, Schmid M: Sex chromosome loss and aging: in situ hybridization studies on human interphase nuclei. Am J Hum Genet 57:1143-1150 (1995).
2. Holmes RI, Keating MJ, Cork A, Trujillo JM, McCredie KB, Freireich EJ: Loss of the Y chromosome in acute myelogenous leukemia: a report of 13 patients. Cancer Genet Cytogenet 17:269-278 (1985).