Anemia de Fanconi: el síndrome de los cromosomas de cristal

Dr. Jordi Surrallés
Catedrático de Genética
Universitat Autònoma de Barcelona
Genome Instability Group: http://gig.uab.cat

Servei d’Anàlisis de Fragilitat Cromosòmica
www.safcro.uab.cat
LA ANEMIA DE FANCONI

-First described by Guido Fanconi in 1927

-Autosomal recessive (one subtype is X linked)
-A very rare disease with a frequency of ~1/400,000
-highly heterogeneous (genetically and clinically)
Disease evolution

Kutler et al., Blood, 2003
Dr. Eunike Velleuer (Dusseldorf) in action in Barcelona, March 2010
DNA REPAIR
INTEREST GROUP
Xeroderma pigmentosum
Chromosome fragility in Fanconi anemia

Control

G1: 70.8%
G2 + M: 21.8%

Fanconi

G1: 36.2%
G2 + M: 56.4%
OVERLAPPING SYNDROMES

Inherited bone marrow failure syndromes:
Dyskeratosis congenita,
Diamond-Blackfan anemia,
Shwachman-Diamond syndrome,
severe congenital neutropenia,
thrombocytopenia absent radii (TAR) syndrome,
amegakaryocytic thrombocytopenia.

Other overlapping syndromes:
Baller-Gerold syndrome,
Nijmegen breakage syndrome (MMC+)
Rothmund-Thomson syndrome,
Roberts syndrome (MMC+)
Warsaw Breakage syndrome (MMC+)
DK-phocomelia,
VACTERL hydrocephalus syndrome,
Wiskott-Aldrich syndrome
# 15 complementation Groups in Fanconi Anemia

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<tr>
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Common phenotype in all genetic subtypes

FA-A

FA-C
Retrovirus-meditated genetic subtyping

![Graph showing relative survival % against MMC (nM)]
Genetic subtyping of 111 Spanish FA patients

Retroviral subtyping, mutational screening, western blot

- A: 74%
- D1: 1%
- D2: 6%
- E: 4%
- G: 3%
- J: 3%
- Unknown: 8%
- C: 1%

whole exome sequencing
ERCC4/XPF mutations and XPF-deficiency in Fanconi anemia patients (FANCQ)

c.1484_1488delCTCAA

c.2065C>A

c.689T>C

c.2371_2398dup28

Am J Hum Genet 2003
XPF cDNA genetically complements MMC sensitivity of FA104 lymphoblasts
XPF/ERCC4 mutations lead to three rare disorders: XP, XFE-progeria, and FA (FA-Q)

Xeroderma pigmentosum  
XFE-progeria  
Fanconi anemia

FANCQ alias for XPF
Interstrand crosslink repair (ICLR)

- Fork stalling
- Unhooking
- Homologous recombination

Nucleotide excision repair (NER)

- UV recognition
- Dual incision
- Excision
- DNA synthesis
- Ligation
1 gene (XPF), 2 repair pathways, 3 syndromes

Xeroderma pigmentosum
Skin cancer

Fanconi anemia
Leukemia and SCC

Progeria
Cancer?

Bogliolo et al., Am J Hum Genet 2013
## 16 complementation Groups in Fanconi Anemia

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Spanish FANCA mutational spectrum

130 mutations, 52 different, 20 novel

Callén et al., 2005; Blood; Kalb et al, Am J Hum Genet 2007; Castella et al., Blood 2011
Cancer pedigree due to mutations in BRIP1/FANCJ found by exome sequencing
Saviour babies: embryo selection for HLA matched

Molly Nash
Preimplantational genetic diagnosis with HLA-matching selection: savior babies
(38 cycles, 7 families)

524 oocytes
299 embryos
75 healthy
26 HLA compatible
16 transferred to uterus
5 implanted (pregnancy)
1 born
Barcelona, August 15th 2006
BON NADAL!
Bone marrow transplant increases cancer risk (SCC)

J Natl Cancer Institute, 2008
Gene therapy: Genetic Correction of Hematopoietic Stem Cells from Patients with Fanconi anemia

Transduction with therapeutic lentiviral vectors

Blood

CD34+ cells Selection

Infusion of transduced graft

Transduction with therapeutic lentiviral vectors
“Natural” vs “medical” gene therapy

Mutation

Gene therapy

Back mutation (mosaics)
Mosaicism

FA patient

FA patient

Mosaic FA patient

Back mutation

Clonal expansion

DEB test: positive

DEB test: positive

DEB test: ?
Mosaicism often results in clinical improvement: “natural” gene therapy
Gene therapy of FANCD1 KO mice (BRCA2−/−)

Figure 3

Donor Chimerism (%) vs. Months after Bone Marrow Transplantation

- FA-D1 Males
- FA-D1 Females

+ EGFP-LVs
+ BRCA2- LVs

3 Gy

#3
#1
#4
#5
#6
#7
#8
#9
Gene therapy of FANCD1 KO mice (BRCA2-/-)

A 2 months after BMT

![Graph showing CFCs Survival (%) vs. MMC (nM)]

B 6 months after BMT

![Graph showing CFCs Survival (%) vs. MMC (nM)]

B

WT FA-D1+ EGFP-LV FA-D1+ BRCA2-LV

![Histogram of % Aberrant Cells](image1)

- MMC + MMC
Phase I/II Gene therapy trial of Fanconi anemia patients with a new Orphan Drug consisting of a lentiviral vector carrying the FANCA gene: A Coordinated International Action

Coordinator: Juan Bueren (Madrid)
WP1: To determine the genetic and hematopoietic characteristics of FA patients.

- Fanconi anemia diagnosis.
- Diagnosis of the pathogenic mutations.
- Early diagnosis of myelodysplastic syndromes or leukemia.
- Diagnosis of mosaic patients with revertant mutations accounting for spontaneous hematological recovery.
- Subtyping of Fanconi anemia patients.
- Prediction of the hematopoietic reserve of the patients.
WP2: To assess the safety and efficacy of an improved mobilization and HSC collection method based on a new mobilization regimen for FA patients with plerixafor and filgrastim.

**AIM:** To collect $4 \times 10^6 \text{ CD34}^+ \text{ cells} / \text{ kg of weight projected to 5 years}$.

- **HSC mobilization:** Filgrastim (10-12 μg / kg every 12 hours) for up to 7 days and plerixafor (240 μg / kg) up to 4 days, 6 to 11 hours before starting apheresis.
WP3: To validate the safety and efficacy of the therapeutic clinical-grade lentiviral vector

Antecedents:

Orphan Medicinal Product Designation: EU 3/10/822
Lentiviral vector containing the Fanconi anemia A (FANCA) gene
WP3: To validate the safety and efficacy of the therapeutic clinical-grade lentiviral vector

AIMs:

- Production of the therapeutic vector under GMP conditions.

- Manufacturing of the medicinal product, genetically modified FA-A CD34+ cells, under GMP conditions.

- Validation of the safety of the medicinal product.
AIM

To demonstrate the safety and obtain the first evidences of clinical efficacy associated to the infusion of the medicinal product: Genetically modified autologous CD34+ cells.

**Inclusion Criteria**

- Patients complementation group: FA-A
  - Moderate to severe aplasia

**Exclusion Criteria**

- Patients with a HLA-identical related donor
- Nº of cryopreserved or fresh CD34+ cells: $<10^5$ CD34+/kg weight
  - Evidence of CD34+ cells transformation
- Evidence of somatic mosaicism in HSCs associated with hematological improvement

WP4: To assess the safety and efficacy of the infusion of CD34+ cells in FA patients, after transduction with the therapeutic lentiviral vector.
Future
Disease-corrected haematopoietic progenitors from Fanconi anemia induced pluripotent stem (iPS) cells

mutation repair

double-strand break (DSB)

save integration (save harbour)
acknowledgements:

Juan Bueren (CIEMAT-Madrid)
Red Española de Anemia de Fanconii
Juan Carlos Izpisua-Belmonte (CMRB-Barcelona)
Javier Benítez (CNIO-Madrid)
Sheila Zúñiga (Sistemas Genómicos, Valencia)
Arleen Auerbach (RU-New York)
Ruud Brakenhoff (VUMC-Amsterdam)
Detlev Schindler (Uni. Wursburg)
Johan de Winter (VUMC-Amsterdam)
Orlando Scharer (SBU-New York)
Koos Jaspers (EU-Rotterdam)
Surrallés’ lab, Barcelona, Spain

Dr. M. Bogliolo
Dr. L. Mina
Dr. J. Minguillón
Dr. R. Pujol
Dr. MJ Ramírez
Dr. J. Surrallés
Dr. G. Hernández
Dr. M. Aza-Carmona
A. Molina
J.P. Trujillo
M. Marin
H. Montanuy
S. Sánchez

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