Constitutional 9p22q translocation in a patient with melanoma, deafness and DNA repair deficiency disrupts p14arf and down-regulates TBX1

Xiaohui Tan1, Sarah Anzick2, Sikandar G. Khan1, Takahiro Ueda1,3, Gary Stone2, John J. DiGiovanna1,4, Deborah Tamura1, Daniel Wattendorf5, Carmen Brewer6, Chris Zalewski6, Robert Walker2, John A Butman7, Andrew Griffith6, Paul Meltzer2, Paul Bergstresser8 and Kenneth H. Kraemer1

1DNA Repair Section, Dermatology Branch, CCR, NCI
2Genetics Branch, NCI
3Pharm/ Medical Device Agency, Tokyo, Japan
4Derm, Brown Med School, Providence, RI
5Office of the Air Force Surgeon General
6Otolaryngology Br, NIDCD
7Radiology, CC
8Derm, Univ of Texas SW, Dallas, TX
DNA REPAIR DEFICIENCY, MELANOMA, DEAFNESS, AND CHROMOSOME 9p22q TRANSLOCATION – A NEW SYNDROME?

Patient DD129BE
Sensorineural deafness,
DiGeorge Syndrome / Velo-cardio-facial Syndrome like features

Melanoma 0.25 mm

Low set ears

Contractures

Posterior fifth toes

12 y/o

14 y/o
CLINICAL EXAMINATION

- **Audiologic assessment**
  No measureable hearing bilaterally and absent vestibular reactivity

- **CT and MRI**
  Bilaterally symmetric cochlear hypoplasia and vestibular dysplasia with absence of the cochlear nerve.
KARYOTYPE ANALYSIS

Reciprocal translocation between the short arm of chromosome 9 and the long arm of chromosome 22

\[ t(9;22)(p21;q11.2) \]
THE AIM

1. To map the chromosome breakpoints in cells from patient DD129BE
2. To develop a detailed molecular characterization of the candidate genes for his clinical syndrome of melanoma, defective DNA repair and deafness.
FISH analysis of t(9;22) with whole chromosome painting

CHROMOSOME 9 and 22 PROBES ARE SPLIT BY THE TRANSLOCATION
FISH analysis of the translocation on chr9

CHROMOSOME 9 p16 PROBE IS SPLIT BY THE TRANSLOCATION
Array Comparative Genome Hybridization (aCGH) of Total Genomic DNA

No detectable changes
BIVARIATE FLOW SORTING TO ISOLATE DERIVATIVE CHROMOSOMES

Derivative Chromosomes can be Separated By Bivariate Flow Sorting

Normal chromosomes

Patient chromosomes

derivative chromosome 9

derivative chromosome 22
Breakpoint Located Within CDKN2A Gene Using aCGH On Flow Sorted Derivative Chromosome 9

Area 1 derivative chromosome 9

Break on Chromosome 9 splits CDKN2A gene
The CDKN2a (p16\textsuperscript{ink4a}), CDKN2b (p15\textsuperscript{ink4b}) Locus on Chromosome 9p21

Exon: 1 (2.5kb) 2 (11.5kb) 1β (19.3kb) 1α (3.4kb) 2 (2.6kb) 3

CDKN2b

\underline{p15^{ink4b}}

p14\textsuperscript{ARF}

CDKN2a

\underline{p16^{ink4a}}

Cyclin D

CDK 4/6

The p53 Pathway

(p53) hdm2 mediated degradation of p53

The pRB Pathway

pRB

E2FDP

E2FDP

Breakpoint on Chromosome 9 Interrupts p14/ARF Protein Leading to Melanoma
Two UV-type Missense Mutations in Exon 1 of $\beta$ of p14arf Gene

Wild type

gCAG$\rightarrow$CGG  pQ57R

gAGA$\rightarrow$GGA  pR62G
The melanoma in patient DD129BE may have resulted when one copy of \textit{p14arf} was disrupted by the translocation followed by somatic mutations in the other copy.
Reduced HCR is not corrected by any of the known XP complementation groups.
Increased HCR by Co-transfection with p14

DNA repair defect improved by transfection with p14 cDNA vector
FISH Analysis of DiGeorge Syndrome Critical Region on Chr22

3 megabase DiGeorge Syndrome Critical Region

VCFS probe

562F10, 3090o16, and 586I18 probes are split by translocation

Normal 22
Der 22

Normal 9
Der 9

Der 22
Normal 22

Der 9
Normal 22
Areas 3-8 pooled derivative chromosome 22

CHROMOSOME 22 BREAKPOINT LIES WITHIN A BREAKPOINT HOTSPOT REGION IN A GAP IN THE HUMAN GENOME MAP

Array CGH On Flow Sorted Derivative 22

BACs

138C22 3148D21 586I18 562F10 278E23

Alignments

17500000 18000000 18500000 19000000 19500000

Breakpoint hotspot

PATRR22(1kb)

Breakpoints

22d 22c 22b 22c 22a

980kb

22q11.2

PATRR22

GAP

GAP

VCFS probe

pooled derivative chromosome 22

CHROMOSOME 22 BREAKPOINT LIES WITHIN A BREAKPOINT HOTSPOT REGION IN A GAP IN THE HUMAN GENOME MAP
Breakpoint Junction is present only in cells from patient DD129BE.
**Junction Fragment Sequences of Der9**

**Chr 9**
- Chr9-1R
- Chr9-5R
- Chr9-6R

**Der 9**
- Chr9-1R
- Chr9-5R
- Chr9-6R

**SINE (88% similarity to regions on PATRR22)**

**TA repeat variable region on chr 22**

**Palindrome in PATRR22**

**TA-rich region flanking the PATRR22**

**SINE (88% similarity to regions on PATRR22 and chr9)**

**TRANSLOCATION BREAKPOINT LIES WITHIN AT-RICH REGIONS ON CHR 9 AND CHR 22**

**Sequence of normal chromosome 9**

**Sequence of der 9**

**Bold lettering indicates the sequence on der(9)**
Localization of Translocation Breakpoints Within PATRR22

\( t(11;22) \)

9,22 TRANSLOCATION BREAKPOINT IS IN A BREAKPOINT HOTSPOT REGION WITHIN PATRR22
TBX1 is 800kb away from the breakpoint in the gap.
FISH analysis of the translocation on chr22 with TBX1 probe

TBX1 PROBE IS NOT SPLIT BY THE TRANSLOCATION
No mutations in promoter or exons in three isoforms of TBX1
Dramatically reduced TBX1 Expression in Patient’s cells

Semiquantitative RT-PCT

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No detectable *TBX1* expression in DD129BE cells by PCR or qRT-PCR

Real Time T-PCT

- **OD129BE**
- **GM16733**
- **GM16735**
Microarray Analysis of Expression of the Genes Around the Translocation

Low expression of CDKN2A and TBX1 by microarray
We have demonstrated that the constitutional t(9;22) translocation occurs in AT-rich repeat intervals on both chromosomes 9 and 22.

The rearrangement was mediated by a 6 bp CACGTG palindromic sequence between the breakpoints. Chromosome 9 had a deletion of 71 bp in an AT-rich repeat region in intron 1 of CDKN2A gene, and chromosome 22 had a deletion of 62 bp in PATRR22 in an uncloned gap.

The melanoma in this patient may have arisen according to the Knudson model in which one copy of p14arf is disrupted by the translocation followed by a somatic mutations in the other copy.
These rearrangements on chromosomes 9 and 22 in patient DD129BE resulted in:

1. down regulation of \textit{CDKN2A} on chr 9 that contributes to the melanoma susceptibility and reduced DNA repair
2. down regulation of \textit{TBX1} on chr 22 that contributes to his deafness.