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Cytogenetic and molecular study of the *PRDX4* gene in a translocation (X;18)(p22;q23) – a cautionary tale

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Abstract

The *PRDX4* gene located at Xp22 codes for a member of the peroxiredoxin gene family. Genes within this family exhibit thioredoxin-dependent peroxidase activity and have been implicated in cellular functioning, including proliferation and differentiation. Recently, *PRDX4* has been identified as a partner gene in an X;21 translocation in a patient with acute myeloid leukemia (AML). To determine whether *PRDX4* was involved in other translocations, leukemia cells from fifteen patients with Xp22 abnormalities were screened for the gene's involvement using fluorescence in situ hybridization (FISH). One sample from a 41 year old female with acute lymphoblastic leukemia (ALL) showed three signals when hybridized with the *PRDX4* probe. Cytogenetic analysis of the sample had identified a t(X;18)(p22,q23). Assuming that the three signals indicated a break within the *PRDX4* gene, we performed FISH experiments and successfully narrowed the breakpoint on chromosome 18 to a 50 kB region. Subsequent analysis using spectral karyotyping showed that the leukemic cells had undergone multiple rearrangements and that a third X chromosome, albeit rearranged, was present. Additional FISH experiments revealed that the third *PRDX4* signal was the result of a third copy of the gene. Analysis of the other rearrangements has helped to characterize the multiple abnormalities within the leukemic cells. Our findings are significant because they underline the importance of using multiple techniques when analyzing complex chromosomal rearrangements in malignant cells.

1) Introduction

Chromosome translocations are known to be an important factor in causing a wide range of cancers, especially leukemia [1]. Although most common translocations have been identified and the breakpoints have been cloned, many rare translocations remain to be detected. Many of the genes cloned from translocation breakpoints have been shown to be critical in malignant transformation; thus identifying and cloning these new breakpoints is important to increase our understanding of genetic changes in leukemic cells. We recently cloned a novel breakpoint in a t(X;21)(p22;q22) translocation, which involved the *AML1* gene on 21 and the *PRDX4* gene on chromosome Xp22 that had not previously been identified in translocations in leukemia [2]. Peroxiredoxins play many functional roles in the cell including proliferation, differentiation, and cell signaling [3]. In the t(X;21)(p22;q22) the 5′ portion of *AML1* is fused

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to the 3′ portion of *PRDX4*. It is very possible that this fusion has a dominant negative effect on the wild type *AML1* and may also lead to loss of *PRDX4* function. This was the first example of antioxidant enzyme involvement in a chromosome translocation in leukemia. Our present study was performed to determine if *PRDX4* was also involved in other translocations in patients with leukemia.

2) Case Report

Fifteen patients with leukemia and Xp22 translocations on cytogenetic analysis were screened using fluorescence in situ hybridization (FISH) with a 173 kb BAC probe covering the *PRDX4* gene (19 kb) (RP1140P7 location 23, 464, 482 to 23, 637, 849). 14 patients showed normal results for the hybridization. A third FISH signal was observed in one patient implicating *PRDX4* involvement.

The patient was a 39 year old Caucasian female who was diagnosed with Pro-B acute lymphoblastic leukemia (ALL) in 2001. She initially presented with a six-week history of fatigue and petechiae and was found to be pancytopenic (WBC 5.4×10^9/l, hemoglobin 9.6 g/dL and platelets 11×10^9/l). A bone marrow biopsy was performed and showed 95% blasts, positive for CD19, CD79 and TdT. The ISCN-karyotype on presentation was 46,XX,t(4;11)(q21;q23) [73%]. The patient was started on chemotherapy per protocol CALGB 19802 [4]. She underwent induction therapy with daunorubicin, cyclophosphamide, vincristine, PEG-asparaginase and prednisone and obtained complete remission (CR). After 5 months of maintenance therapy with Vincristine, MTX, prednisone, 6-mercaptopurin she relapsed in 2002. Her bone marrow had 48% blasts and in a repeat cytogenetic analysis showed the t(4;11)(q21;q23). After off-protocol treatment with ifosfamide, MTX, vincristine, cytarabine, etoposide and dexamethasone failed to induce a remission, a matched unrelated donor bone marrow transplant was done. After a short CR she relapsed again, this time showing karyotypic evolution, 47,X,t(X;18)(p22;q23), add(1)(q44), t(4;11)(q21;q23), t(6;14)(q23;q21), der(7)t(7;7)(p15,q11),+13, −14, +17, +20 in 60 % of metaphase cells (Fig 1). A second bone marrow transplantation was performed, yielding only a three month period of CR. Cytogenetic analysis of the 3rd relapse in 2003 showed the t(X;18)(p22;q23) in 50 % of metaphase cells. The patient died shortly after that in a hospice.

FISH with BACRP11-40P7 was performed on cells from the bone marrow aspirate on 2nd relapse and showed three signals on hybridization (Fig 2). Assuming that *PRDX4* was involved in this translocation, we tried to identify the location of the breakpoint on 18q22 and the partner gene of the translocation on chromosome18. A 22 Mb region on chromosome 18 was investigated with 44 BAC clones. Eighteen BAC clones mapping from 54.6 Mb to 67.1 Mb on chromosome 18 (RP11-108P20, RP11- 299P2, RP11-727B4, RP11-454J3, RP11-74B10, RP11-563H6, RP11-255L11, RP11-755G16, RP11-704G7, RP11-484N16, CTD-2311I12, CTD-2523K8, RP11-41O4, RP11-433A23, RP11- 792O7, CTD-2593F24, RP1-47G4, RP11-94I6) remained on chromosome 18 indicating that they were proximal to the breakpoint. Twenty-three probes from 67.2 Mb to 76.1 Mb (RP11-510D19, RP11-752P2, RP11-723G8, RP11-702M18, RP11-1061K3, RP11-131K21, RP11-676J15, RP11-396D4, RP11-504I13, RP11-162A12, RP11-751H17, RP11-130J1, RP11-849I19, RP11-345O15, RP11-042B2, RP11-298P14, CTD-2286N8, RP11-1136J12, RP11-800A18, RP11-196B3, RP11-154H12, RP11-680N10, RP11-245M2) translocated to the X chromosome, thus showing that they were telomeric of the breakpoint. The large number of BAC clones used for the location of the breakpoint can be explained in part by incorrect information provided from an online gene database. One of the BAC clones we used in the beginning (RP11-755G16) was initially mapped by the database 1.64 Mb further telomeric on chromosome 18 than its actual location. Thus we initially obtained contradictory results and the identification of the breakpoint was delayed. We notified the database and the erroneous map location was corrected.

Finally the potential breakpoint could be narrowed to a 50 kilobase region between the genes *SOCS6* and *CBLN2* on 18q23. Two BAC clones (RP11-139B13, location 67128221 to 67283891 on 18q22 and RP11-432G11, 67033407 to 67239179) were found that showed a split signal on both the derivative X and 18 chromosome and thus appeared to span the translocation breakpoint. Six uncharacterized expressed sequence tags (ESTs) but no known genes were located in the breakpoint area on chromosome 18.

However, our mapping data lead to unexpected results. When the split chromosome 18 probes were hybridized with the original *PRDX4* probe (RP11-40P7), we did not see a fusion signal on chromosome 18 as expected. Two *PRDX4* signals appeared on the derivative X involved in the translocation with chromosome 18 and on what was thought to be a normal chromosome X. The third *PRDX4* signal appeared on a small unidentified chromosome (Fig 3). To clarify this unexpected result, spectral karyotyping (SKY) was used to identify all chromosomes. SKY analysis showed that the unidentified chromosome with the third *PRDX4* signal was actually the short arm of a third X that appeared in all of the abnormal cells (Fig 4). This X had initially been identified as a third chromosome 17 in the banding karyotype (Fig 1). It appears that the long arm of a second X chromosome was involved in a translocation with chromosome 1 and the residual short arm was involved with chromosome 18.

Now the unresolved question was whether the break on the X chromosome really involved the *PRDX4* gene or a region on the X chromosome further telomeric to the gene. Using two probes RP13 314C10 (Accession number AC131011.5), from 23.64 Mb to 23.81 Mb, and RP11 911N20, from 23,37 Mb to 23,55 Mb on Xp22, that flank *PRDX4*, we determined that *PRDX4* was not involved. Both signals colocalized on all three X chromosomes, proving that the three *PRDX4* signals were a result of a third copy of the gene and not a split within the gene (Fig 5).

3) Discussion

PRDX4 is a member of the peroxiredoxin gene family that exhibits thioredoxin dependent peroxidase activity. All peroxiredoxin genes are highly conserved in eukaryotes and prokaryotes and are ubiquitously expressed [5,6]. *PRDX4* was shown to play a role in tumor suppression and is significantly down regulated in specific leukemia subtypes [7,8]. Recently, altered expression of *PRDX4* was also shown in stomach and colon cancer metastases [9,10]. After we reported on the involvement of *PRDX4* in a translocation of an AML patient [2], we wanted to determine if this gene might also be involved in other leukemia translocations. The split FISH signal in an ALL patient with a t (X;18)(p22;q23) using a probe spanning *PRDX4* implied that *PRDX4* was involved in the translocation. However, this assumption was disproved with further FISH experiments. Using BAC probes that span the breakpoint on chromosome 18 and the *PRDX4* probe, no fusion signal was seen on the derivative chromosome 18. SKY analysis helped to clarify the chromosomal abnormalities in the leukemic cells. These abnormalities include the presence of an additional X chromosome in the abnormal cells, explaining the three signals for the *PRDX4* probe, and further that this third X chromosome had undergone additional rearrangement with the long arm of chromosome 1. The initial cytogenetic analysis of the karyotype did not identify these chromosomal changes. Although karyotyping with chromosomal banding is the standard method for identifying chromosomal aberrations, it appears that highly rearranged karyotypes are often difficult to interpret with conventional cytogenetics alone. Studies have demonstrated that SKY analysis could substantially improve the accurate assessment of genetic abnormalities in malignant cells and even correct the cytogenetic interpretation [11–13]. Our additional investigation of the karyotypic changes were crucial to obtain the correct analysis and they stress that the use of multiple techniques in malignant cells is required to confirm complex rearrangements. Without

these additional studies the complex rearrangements that are likely responsible for the malignant behavior of this leukemia would not have been properly identified.

The (X;18) translocation in our patient appeared as a secondary chromosomal aberration in addition to the known t(4;11) after relapse following bone marrow transplantation. Additional aberrations do not have an impact on survival or prognosis in patients with 11q23 abnormalities; however the characterization of these changes is important to determine their potential role in the evolution of *MLL*-driven leukemogenesis [14–16].

Conclusions

We conclude that combining different techniques, i.e. conventional chromosomal banding analysis, FISH and SKY provides the most comprehensive cytogenetic approach. SKY was shown again to help to clarify the chromosomal rearrangements in complex karyotypes.

The two BAC probes flanking *PRDX4* finally confirmed that this gene was not involved in the translocation. Our initial assumption that the original split of BACRP11-40P7 meant involvement of *PRDX4* can be explained by the larger size of this clone (173 kb) compared to *PRDX4* (19 kb).

Considering the incorrect database mapping of one of our BAC clones, our study can also be seen as an alert to other groups that one should not always rely on the information from a single online database.

Acknowledgements

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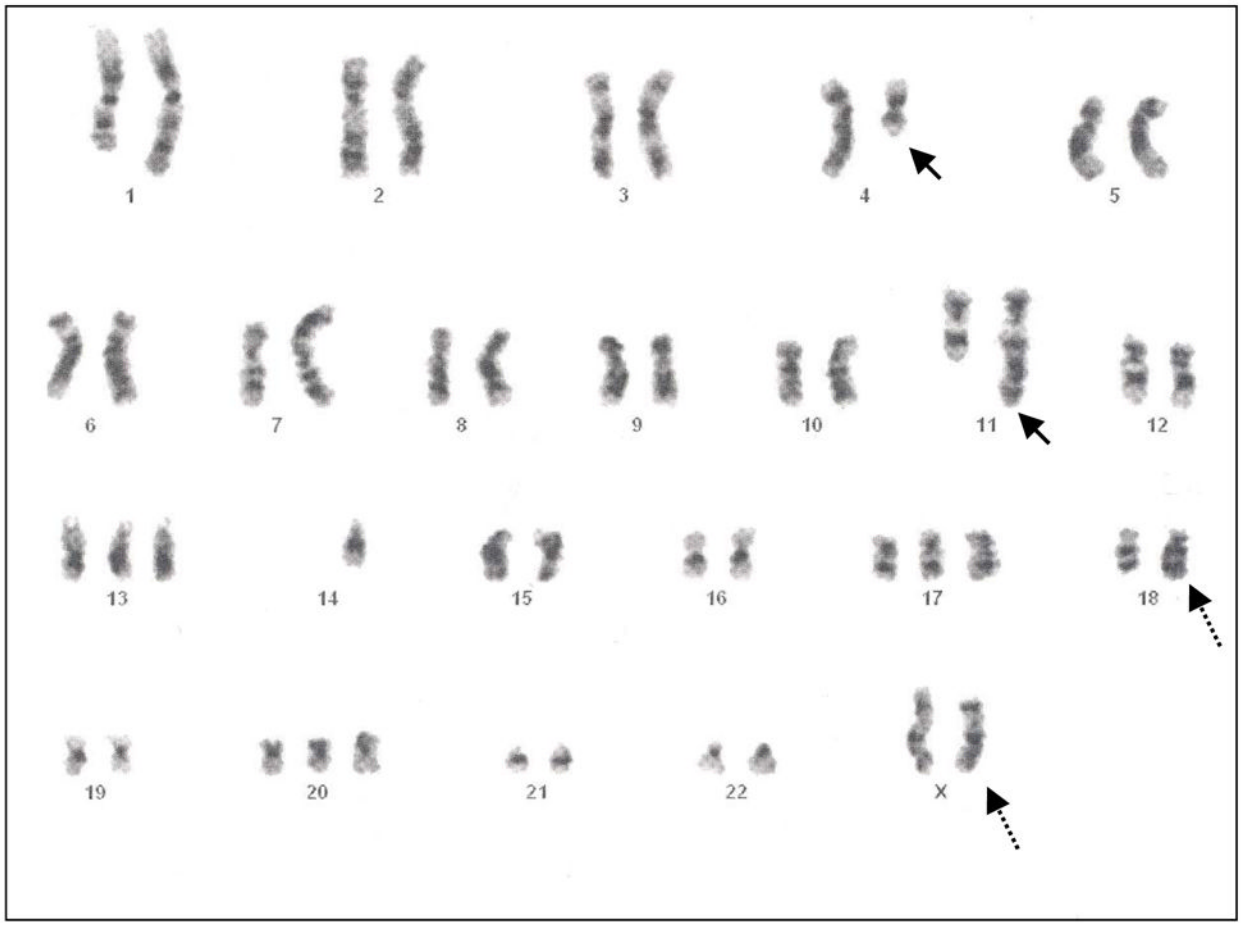

Figure 1.
Karyotype after second relapse, interpreted as 48,X, t(X;18)(p22;q23), add (1)(q44), t(4;11)(q21;q23), t(6;14)(q23;q21), der(7)(t7;7)(p15;q11), +13, −14, +17, +20 [60%]. The derivative chromosome 4 and 11 are indicated with black arrows, X and 18 with dashed-black arrows. The third chromosome 17 was shown to be a der(x) chromosome by spectral karyotyping.

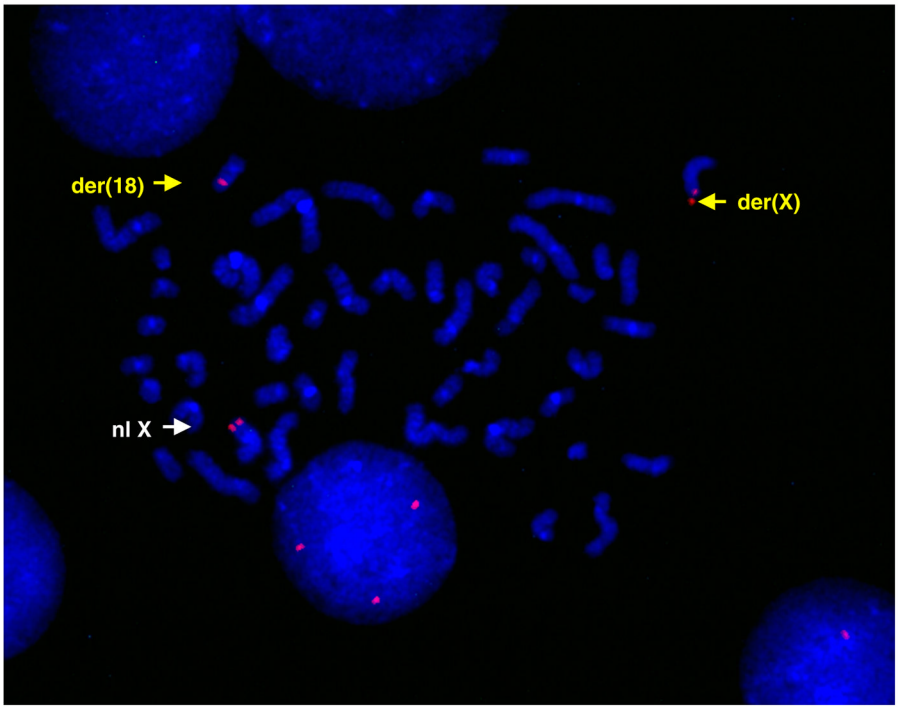

Figure 2.
Hybridization with BAC RP11-40P7, labeled with Spectrum® orange. Signals were seen on the normal X, the derivative X and a chromosome that was initially interpreted as the derivative chromosome 18.

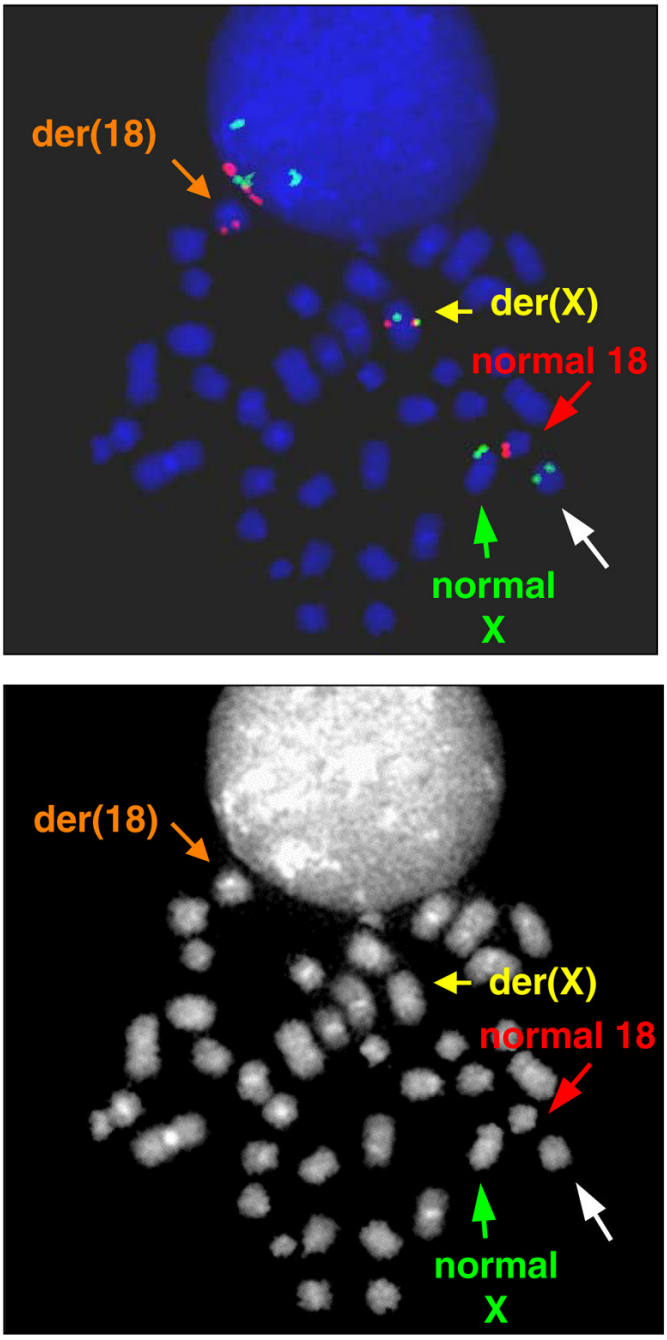

Figure 3.
BAC RP11-40P7 spanning *PRDX4* was labeled with Spectrum® green, RP11- 432 G11 with Spectrum® orange. Both normal chromosome 18 and X show one normal signal, respectively. The derivative X chromosome shows the expected fusion signal, while the derivative chromosome 18 shows only one red signal. The third green signal for *PRDX4* appears to be on a then unidentified chromosome, indicated with a white arrow.

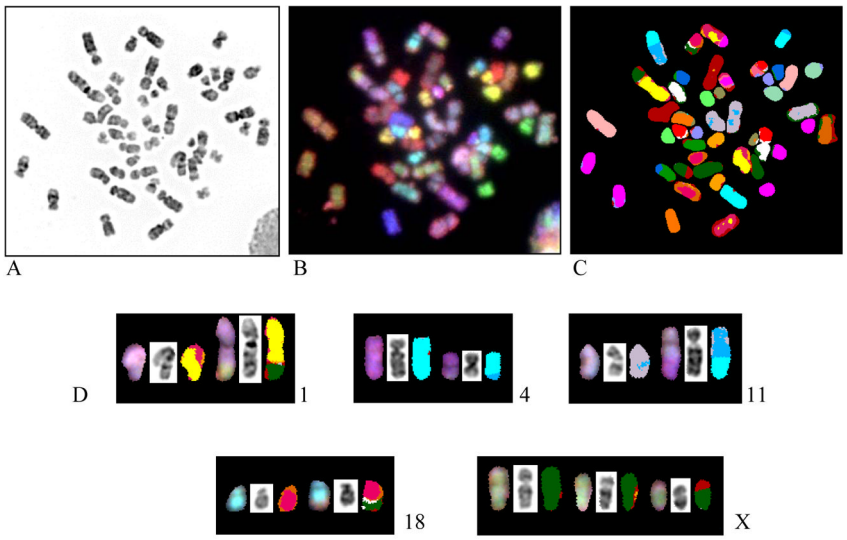

Figure 4.
SKY Image and partial karyotype. A: DAPI stained metaphase cell, B: Spectral image, C: Classified image, D: Partial karyotype for chromosomes 1, 4, 11, 18 and X.

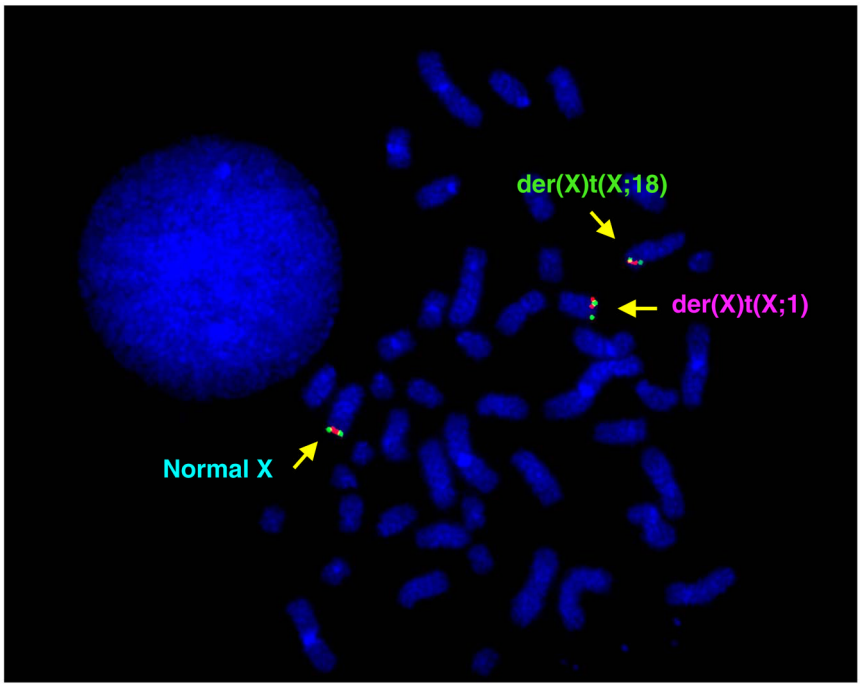

Figure 5.
FISH using two probes flanking the *PRDX4* gene. The centromeric probe RP13 314C10 is labeled with Spectrum® green, the telomeric probe (RP11-911N20) with Spectrum® orange. Both probes colocalize on all three X chromosomes indicating that *PRDX4* is not split by the translocation.