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Changes in Nuclear Orientation Patterns of Chromosome 11 during Mouse Plasmacytoma Development[1]

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Abstract

Studying changes in nuclear architecture is a unique approach toward the understanding of nuclear remodeling during tumor development. One aspect of nuclear architecture is the orientation of chromosomes in the three-dimensional nuclear space. We studied mouse chromosome 11 in lymphocytes of [T38HxBALB/c]N mice with a reciprocal translocation between chromosome X and 11 (T38HT(X;11)) exhibiting a long chromosome T(11;X) and a short chromosome T(X;11) and in fast-onset plasmacytomas (PCTs) induced in the same strain. We determined the three-dimensional orientation of chromosome 11 using a mouse chromosome 11 specific multicolor banding probe. We also examined the nuclear position of the small translocation chromosome T(X;11) which contains cytoband 11E2 and parts of E1. Chromosomes can point either with their centromeric or with their telomeric end toward the nuclear center or periphery, or their position is found in parallel to the nuclear border. In T38HT(X;11) nuclei, the most frequently observed orientation pattern was with both chromosomes 11 in parallel to the nuclear border ("PP"). PCT cells showed nuclei with two or more copies of chromosome 11. In PCTs, the most frequent orientation pattern was with one chromosome in parallel and the other pointing with its centromeric end toward the nuclear periphery ("CP"). There is a significant difference between the orientation patterns observed in T38HT(X;11) and in PCT nuclei ($P < .0001$).

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Introduction

Chromosomes are organized in evolutionary conserved chromosome territories [1]. Their nonrandom three-dimensional (3D) positions were previously described [2], e.g., the localization of the active and inactive chromosome X and their respective genes [3,4]. Euchromatin of rod photoreceptor cells in nocturnal mammals is found in the periphery, whereas it is found in the center in diurnal mammals [5]. Not only chromosome territories are in the focus of research but also the localization of telomeric regions [6,7].

Tumor development is greatly influenced by genomic instability [8], and telomere dysfunction plays an important role in genomic instability [9]. Therefore, it is essential to study nuclear architecture in normal and tumor cells. Movement of telomeric regions during the cell cycle was observed in living ECV-TRF1 and -TRF2 cells [10] and in human osteosarcoma U2OS cells [11]. Chromosomes of primary human fibroblasts alter their positions within 15 minutes after they are made quiescent due to a removal of serum from the culture medium. This repositioning is probably dependent on nuclear myosin 1β [12]. Further changes of chromosome positions can be found during adipocyte differentiation [13] or T-cell differentiation [14].

Telomere lengthening is a method to prevent genomic instability of rapidly dividing cells [15]. This can occur due to telomerase [16] or due to cycles of homologous recombination during the process of

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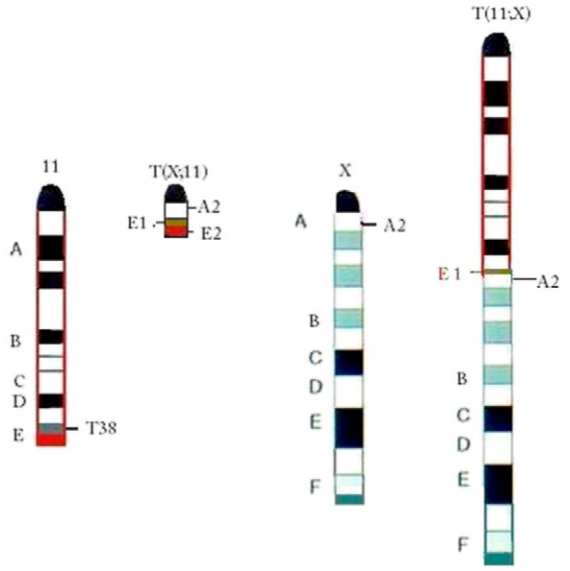

Figure 1. Graphical illustration of the chromosomal constitution of chromosomes 11 in the (BALB/c x T38H) F1 N backcross generation mouse. Chromosome (Chr) 11 with the breakpoint T38H in the telomeric cytoband 11E1 (brown), while the breakpoint in Chr X is located in the centromeric A2 band. The cytoband 11 E2 is colored in red. The reciprocally translocted T(11;X) chromosome resulted from the fusion of the ABCD bands of Chr 11 proximal to the T38H breakpoint with the centromeric A2 band of Chr X. The T(X;11) chromosome was generated by the translocation of the X-derived A2 sub-band onto the 11E1 cytoband of Chr 11. This figure has been published in *Genes Cancer*. 2010;1(8):847–858 [19] and is reprinted here with permission.

alternative telomere lengthening [17]. In fast-onset plasmacytomas (PCTs), the telomere length is significantly increased for the translocation chromosome T(X;11) carrying 11E2 [18].

In the current study, we used a [T38HxBALB/c]N congenic mouse model with a reciprocal translocation between chromosomes X and 11 (rcpT(X;11). This unique mouse model exhibits a long chromosome T(11;X) and a short chromosome T(X;11). The short chromosome T(X;11) contains cytoband 11E2 and parts of cytoband E1 (Figure 1) [19]. To determine the chromosome orientation in cancer cells and in the same cell lineage, we studied mouse PCT induced in this unique mouse model. There are slow- and fast-developing PCTs. Slow-onset PCTs are induced only by pristane (2,6,10,14-tetramethylpentadecane) [20]; fast-onset PCTs are induced by pristane and *v-abl/myc* [19,21]. In the current study, we focused on the fast-onset PCTs. These exhibit a nonrandom duplication of chromosome 11, cytoband 11E2, associated with the overexpression of genes within 11E2 [19]. Cytoband 11E2 is syntenic to human chromosome 17q25 and rat 10q32 [22]. It is frequently altered in tumors of lymphoid and nonlymphoid origin [23–25]. The mean latency of fast-onset PCTs is only 45 days [19,21]. We compared these fast-onset PCT cells with control B lymphocytes of [T38HxBALB/c]N mice with the rcpT(X;11) translocation (T38HT(X;11)).

Our aim was to determine the orientation of chromosome 11 in PCTs and lymphocytes of [T38HxBALB/c]N rcpT(X;11) mice. In a previous study, we determined the orientation of chromosome 11 in 3D nuclei of PreB lymphocytes of BALB/c origin and of [T38HxBALB/c]N wild-type mice without the rcpT(X;11) translocation [26] and found a distinct difference between the frequency of the observed orientation patterns in both cell types. Both normal lymphocyte types studied showed a preference in chromosome 11 orientation, where both chromosomes 11 were observed in parallel to the nuclear border [26]. In the current study, we investigated potential changes in the orientation that occur during the process of PCT development.

Originally, multicolor banding (mBANDing) was developed to detect intrachromosomal changes in metaphases [27]. In our previous study, we used mBANDing for the first time in 3D interphase nuclei to determine the orientation of chromosome 11. Chromosome 11 is labeled by four overlapping fluorochromes (Texas Red, GOLD, DEAC, and FITC). This enabled us to analyze whether the centromeric or the telomeric end was orientated toward the nuclear center or periphery. Only one other group used mBANDing on interphase nuclei before. They studied the grade of condensation of human chromosome 5 [28].

We analyzed the orientation patterns of chromosome 11 in PCT cells and [T38HxBALB/c]N rcpT(X;11) lymphocytes. There was a significant difference noted with respect to their chromosome 11 orientation ($P < .0001$). The nuclear position of the small translocation T(X;11) was also studied visually. It was most frequently found in the intermediate region of the nucleus. There was no significant change in position of T(X;11) detected between the two cell types ($P = .06$).

Material and Methods

Cell Harvest

Primary lymphocytes were harvested from spleens of 6- to 8-week-old congenic [T38HxBALB/c]N rcpT(X;11) mice [19]. PCT cells were harvested from the ascites of fast-onset PCT mice. The [T38HxBALB/c]N rcpT(X;11) mice were pretreated with pristane intraperitoneally and after 5 days infected with a *v-abl/myc* virus also administered intraperitoneally. The mean latency of fast-onset PCTs is 45 days [19,21]. Procedures were performed in accordance to Animal Protocol 11-019 approved by Central Animal Care Services, University of Manitoba (Winnipeg, MB, Canada).

3D Nuclear Hybridizations

For 3D nuclei fixation, lymphocytes were centrifuged at 1000 rpm for 10 minutes. After resuspension of the pellet, cells were carefully placed onto slides and fixed with 3.7% formaldehyde/1× PBS for 20 minutes at room temperature. Next, the slides underwent washing steps in 1× PBS shaking. Subsequently, the slides were washed in 0.5% Triton-X-100 for 10 minutes. The slides were incubated for 1 to 2 hours in 20% glycerol and were then subjected to four freeze-thaw cycles in liquid nitrogen afterward. Next, the slides were washed 3× in 1× PBS and then incubated in fresh 0.1 M HCl for 5 minutes. After washing the slides in 1× PBS, they were placed for at least 1 hour in 70% formamide/2× SSC.

Multicolor Banding

The mBANDing probe for mouse chromosome 11 (Metasystems, Altussheim, Germany) was developed by Benedek et al. (2004) [29]. The slides were equilibrated in 2× SSC, treated with RNAase A (100 µg/ml) in 2× SSC at 37°C for 1 hour, and then incubated in freshly prepared 0.01 M HCl with 100 µg/ml pepsin for 2 minutes. After washing the slides in 1× PBS, they were pretreated in 1% formaldehyde in 1× PBS/50 mM $MgCl_2$, followed by washing in 1× PBS. Next, the slides were incubated in 0.1× SSC and then