‘Mitotic drive’ of expanded CTG repeats in myotonic dystrophy type 1 (DM1)

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In myotonic dystrophy type 1 (DM1), an expanded CTG repeat shows repeat size instability in somatic and germ line tissues with a strong bias toward further expansion. To investigate the mechanism of this expansion bias, 29 DM1 and six normal lymphoblastoid cell lines (LBCLs) were single-cell cloned from blood cells of 18 DM1 patients and six normal subjects. In all 29 cell lines, the expanded CTG repeat alleles gradually shifted toward further expansion by ‘step-wise’ mutations. Of these 29 cell lines, eight yielded a rapidly proliferating mutant with a gain of large repeat size that became the major allele population, eventually replacing the progenitor allele population. By mixing cell lines with different repeat expansions, we found that cells with larger CTG repeat expansion had a growth advantage over those with smaller expansions in culture. This growth advantage was attributable to increased cell proliferation mediated by Erk1,2 activation, which is negatively regulated by p21WAF1. This phenomenon, which we designated ‘mitotic drive’, is a novel mechanism which can explain the expansion bias observed in DM1 PBLs, based on variable repeat sizes among different tissues (9).

The expanded CTG repeat in peripheral blood leukocytes (PBLs) is unstable throughout the life of a patient with DM1, with gradual increases in the average repeat size and the repeat size heterogeneity with age (7). Based on these observations, Monckton et al. (8) have proposed a model for the somatic instability of the DM1 CTG repeat. In this model, somatic instability proceeds through a directional pathway which involves ‘step-wise’ gains of a small number of repeat units; thus, as the subject ages, the allele distribution gradually shifts towards expansion with increasing repeat size heterogeneity. Thus, somatic instability of the DM1 CTG repeat involves a dynamic process in which the repeat size increases with age at different rates in various tissues and, therefore, should play important roles in tissue- and age-specific phenotypic variability. To further characterize the somatic instability, we have developed a cell culture system using clonal lymphoblastoid cell lines (LBCLs) prepared from DM1 patients (10). Each clone was derived from a single cell; therefore, any alleles with repeat sizes different from the progenitor allele can be detected as mutant alleles. SP-PCR analyses of these mutant alleles showed two types of mutations in short-term culture: a frequent ‘step-wise’ gain or loss of a small number of repeat units and a ‘gross’ change of repeat size that occurs infrequently with a bias toward contraction (10). In this study, we demonstrate expansion dynamics of these mutations in long-term cultures, and present evidence for a novel mechanism which can explain the expansion bias observed in DM1 PBLs, based on preferential proliferation of cells with larger CTG repeats.

INTRODUCTION

Myotonic dystrophy type 1 (DM1, OMIM 160900) is a progressive multisystemic autosomal dominant disorder which shows a phenomenon known as anticipation (1). Anticipation denotes progressively earlier onset of the disease with increasing severity in successive generations. The mutation of DM1 is an unstable CTG repeat expansion in the 3′ untranslated region of the myotonic dystrophy protein kinase (DMPK) gene on chromosome 19q13.3 (2–4). The repeat size of the expanded allele inversely correlates with the age of disease onset and becomes progressively larger in successive generations in DM1 families, providing a molecular basis of anticipation (1,5). Expanded CTG repeat alleles also show a high level of somatic instability, which is evidenced by a smear on Southern and PCR analyses (6,7). Using small pool PCR (SP-PCR), the smear can be resolved into individual alleles with heterogeneous repeat sizes (8). The somatic instability is also evident as variable repeat sizes among different tissues (9).

The expanded CTG repeat in peripheral blood leukocytes (PBLs) is unstable throughout the life of a patient with DM1, with gradual increases in the average repeat size and the repeat size heterogeneity with age (7). Based on these observations, Monckton et al. (8) have proposed a model for the somatic instability of the DM1 CTG repeat. In this model, somatic instability proceeds through a directional pathway which involves ‘step-wise’ gains of a small number of repeat units; thus, as the subject ages, the allele distribution gradually shifts towards expansion with increasing repeat size heterogeneity. Thus, somatic instability of the DM1 CTG repeat involves a dynamic process in which the repeat size increases with age at different rates in various tissues and, therefore, should play important roles in tissue- and age-specific phenotypic variability. To further characterize the somatic instability, we have developed a cell culture system using clonal lymphoblastoid cell lines (LBCLs) prepared from DM1 patients (10). Each clone was derived from a single cell; therefore, any alleles with repeat sizes different from the progenitor allele can be detected as mutant alleles. SP-PCR analyses of these mutant alleles showed two types of mutations in short-term culture: a frequent ‘step-wise’ gain or loss of a small number of repeat units and a ‘gross’ change of repeat size that occurs infrequently with a bias toward contraction (10). In this study, we demonstrate expansion dynamics of these mutations in long-term cultures, and present evidence for a novel mechanism which can explain the expansion bias observed in DM1 PBLs, based on preferential proliferation of cells with larger CTG repeats.

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RESULTS

Step-wise mutations of expanded CTG repeats toward further expansion in DM1 LBCLs

To investigate the instability of CTG repeat size, we established 22 clonal LBCLs obtained from 18 DM1 patients that showed variable sizes of the expanded CTG repeat (Table 1). Additionally, six lines (0313-1, 0313-2, 0313-7, 0313-11, 0313-15 and 0313-20) and two lines (1008-1-1 and 1008-1-3) were subcloned from a single cell of parental clonal lines, 0313 and 1008-1 (Table 1). Since clonal cells were derived from a single cell, any alleles that deviate from the progenitor allele must have resulted from repeat size mutations in culture. The clones were allowed to grow until the cell number reached $3 \times 10^7$, and $1 \times 10^7$ cells were passed; this process was repeated as long as the cells stayed viable. Normal alleles of DM1 and normal LBCLs were stable throughout the passages. In contrast, expanded CTG repeat alleles in all 29 DM1 LBCLs showed two types of mutations similar to those observed in our previous short-term culture experiments (10); frequently-seen ‘step-wise’ mutations that result in gains and losses of a small number of repeat units around the progenitor allele, and relatively rare ‘gross’ mutations that involve large repeat size changes (Fig. 1). When we compared two consecutive passages using the SP-PCR technique (8), changes in the modal size of the expanded alleles by the step-wise mutations that result in gains and losses of a small number of repeat units around the progenitor allele, and relatively rare ‘gross’ mutations that involve large repeat size changes (Fig. 1). When we compared two consecutive passages using the SP-PCR technique (8), changes in the modal size of the expanded alleles by the step-wise mutations were hardly appreciable, although subtle size heterogeneity of these alleles was evident around the progenitor allele. After several passages, however, we were able to detect a gradual increase of the modal allele size in all cell lines, indicating an expansion bias of the step-wise mutation (Fig. 1) similar to the in vivo model based on the data obtained in PBLs of DM1 patients (8); however, repeat size heterogeneity did not dramatically increase over time in most of these LBCLs, giving rise to the ‘synchronized’ expansion (Figs 1 and 2B) which was previously described in cultured dura mater cells and myoblasts derived from DM1 patients (11).

Gross mutations of expanded CTG repeats toward further expansion in DM1 LBCLs

The gross mutants showed unexpected characteristics. The majority of gross mutants showed contraction of the expanded CTG repeats as we previously reported (10). The gross mutants, which include those with repeat contraction as well as those relatively rare ones with further expansion, mostly disappeared in the following passages (Fig. 1). However, a rapidly proliferating mutant with a gain of large repeat size (by 40–290 repeats) appeared occasionally and became the major allele population that eventually replaced the progenitor allele population (Fig. 3). This phenomenon was observed in eight of the 29 clonal LBCLs (Table 1). Thus, out of eight gross mutants that replaced the progenitor allele population, eight resulted from expansion mutations (the probability of this observation occurring by chance is 1/256 or 0.004), whereas we did not observe contracted gross mutants surviving more than a few passages. These eight included two of the six
Table 1. The 29 clonal LBCLs from 18 DM1 patients, presented in the order of the initial CTG repeat size, and six normal cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Initial (CTG)$_n$</th>
<th>Bimodal phase</th>
<th>Final (CTG)$_n$</th>
<th>$\Delta$ (CTG)$_n$</th>
<th>Max. Pass.</th>
</tr>
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<td></td>
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<tr>
<td>1008-1-3</td>
<td>180</td>
<td>180/470</td>
<td>505</td>
<td>325</td>
<td>14</td>
</tr>
<tr>
<td>1008-1-1</td>
<td>550</td>
<td>–</td>
<td>570</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
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<td>230</td>
<td>–</td>
<td>250</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>0300-2</td>
<td>250</td>
<td>270/600</td>
<td>620</td>
<td>370</td>
<td>7</td>
</tr>
<tr>
<td>1219-2</td>
<td>310</td>
<td>290/330</td>
<td>340</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>0205-1</td>
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<td>–</td>
<td>450</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
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<td>370</td>
<td>370/380</td>
<td>380</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
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<td>370</td>
<td>–</td>
<td>430</td>
<td>60</td>
<td>11</td>
</tr>
<tr>
<td>0113-4</td>
<td>390</td>
<td>–</td>
<td>420</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
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<td>390</td>
<td>–</td>
<td>420</td>
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<td>430</td>
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<td>500/610</td>
<td>630</td>
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<td>–</td>
<td>520</td>
<td>30</td>
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<tr>
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<td>560/580</td>
<td>580</td>
<td>30</td>
<td>11</td>
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<tr>
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<td>–</td>
<td>690</td>
<td>100</td>
<td>8</td>
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<td>–</td>
<td>650</td>
<td>40</td>
<td>12</td>
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<td>630</td>
<td>–</td>
<td>660</td>
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<td>–</td>
<td>780</td>
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<td>11</td>
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<tr>
<td>0454-4</td>
<td>760</td>
<td>–</td>
<td>770</td>
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<td>11</td>
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<td>–</td>
<td>790</td>
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<tr>
<td>0308-4</td>
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<td>–</td>
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<td>10</td>
<td>11</td>
</tr>
<tr>
<td>0102-8</td>
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<td>–</td>
<td>1060</td>
<td>80</td>
<td>9</td>
</tr>
<tr>
<td>0465-7</td>
<td>1000</td>
<td>–</td>
<td>1030</td>
<td>30</td>
<td>8</td>
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<tr>
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<td>1150</td>
<td>–</td>
<td>1180</td>
<td>30</td>
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</tr>
<tr>
<td>0465-1</td>
<td>1280</td>
<td>–</td>
<td>1300</td>
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<tr>
<td><strong>Normal cell lines</strong></td>
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<tr>
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<td>–</td>
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<td>10, 10</td>
<td>0</td>
<td>25+</td>
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<tr>
<td>NL0423-2</td>
<td>5, 10</td>
<td>–</td>
<td>5, 10</td>
<td>0</td>
<td>25+</td>
</tr>
<tr>
<td>NL0424-1</td>
<td>5, 13</td>
<td>–</td>
<td>5, 13</td>
<td>0</td>
<td>25+</td>
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<tr>
<td>NL0425-1</td>
<td>5, 11</td>
<td>–</td>
<td>5, 11</td>
<td>0</td>
<td>25+</td>
</tr>
</tbody>
</table>

The first four digits of the cell line I.D. show the DM1 patient from whom the cell line was derived, the second set of numbers indicates the identity of the primary clone and the third set of numbers identifies subclones derived from clones *1008-1 and *0313-1.

All LBCLs showed step-wise increases in the CTG repeat size.

Eight LBCLs showed an additional gross mutation event with a bimodal transitional phase.

In the LBCLs there are no significant correlations among the initial CTG repeat size, repeat size change and the maximal number of passages.

In the normal cell lines, no expansions were noted. All normal cell lines lived over 25 passages, which is significantly longer than the lifespan of cell lines derived from patients with DM1.
subclonal lines (0313-1-2 and 0313-1-20). The remaining four subclonal lines did not yield such gross mutants. Furthermore, we retrieved the parental line (0313-1) stored frozen at a passage prior to the appearance of the bimodal distribution of the expanded alleles and continued passages. These cells did not reproduce the appearance of the gross mutant peak in subsequent passages. These observations suggest that the appearance of survivable gross mutants was dictated largely by random chance and was, perhaps, not tightly regulated by genomic background. We also found bimodal distributions of the expanded CTG repeat alleles in two patients with the congenital form of DM1, suggesting a potential biological relevance of the gross mutation (Fig. 4A and B). We postulated that this unexpected behavior is due to a growth advantage of the mutant that has a larger CTG repeat expansion over the progenitor cell population. In a cell culture system undergoing multiple passages, cells are subjected to genetic drift and a reduction of the population size at each passage. A cell population that has a relatively high proliferation rate should have a higher probability for increasing its population size in each passage.

‘Mitotic drive’ (growth advantage of DM1 LBCL subclones with larger CTG repeat expansion)

To investigate the hypothesis that DM1 LBCLs with larger CTG repeat expansion have a growth advantage over those with smaller CTG repeat expansion, we used subcloned cell lines carrying different sizes of the expanded CTG repeat but sharing the same genomic background and Epstein–Barr virus (EBV)-genome incorporation pattern. Mixing these subclones in pairs (n = 6) in culture consistently resulted in survival of the subclone with the larger expanded repeat over 3–10 passages (the probability of this observation occurring by chance is 1/64 or 0.016) (Fig. 5). The mixed subclones were in the log phase of cell growth in the same passage. We determined the potential doubling time (Tpot = ln(2) / cell production rate) of these subclones by flow-cytometric analysis of cells pulse-labeled with bromodeoxyuridine (BrdUrd) (12). The subclones studied were in the log phase of the growth in culture after the same number of passages. The size of CTG repeat expansion showed an inverse correlation with cell doubling time (Fig. 6). These data suggest that LBCLs with larger CTG repeat expansion have a growth advantage over those with smaller CTG repeat expansion and that this growth advantage is attributable to the increased cell proliferation rate.

Ras/Erk1,2 activation negatively regulated by p21WAF1 in accelerated proliferation of DM1 LBCLs

In these DM1 LBCL subclones, increased activities (but not expression) of Ras and Erk1,2 were correlated with repeat size and proliferation rate, whereas the p21WAF1 level was inversely correlated with them (Fig. 7A and B). Yet the size of the CTG repeat expansion did not show any correlation with Akt activity (Fig. 7A) nor with Ras, Erk1,2, p53, Bcl-2 or Bax levels (data not shown). Treating these subclones with an antisense oligonucleotide specific for p21WAF1 further increased the activity of Erk1,2 but not Akt, suggesting that p21WAF1 is a negative regulator of Erk1,2 activity (Fig. 8). These observations suggest that the increased proliferation of DM1 LBCLs with larger CTG expansion is mediated by downregulation of p21WAF1 which leads to activation of the Ras-Erk1,2 pathway.
Limited lifespan of DM1 LBCLs

All six normal clonal LBCLs looked viable on phase microscopic examination without noticeable changes in the proliferation rate throughout the extended culture period. Although the DM1 LBCL clones also showed similar viability in culture for most of their lifespan, these cell lines failed to survive in culture after 7–22 passages. During the last one to two passages, the number of non-viable cells gradually increased with slowing growth of viable cells. The lifespan of DM1 LBCL clones and subclones (mean ± standard deviation = 10.5 ± 3.1 passages; n = 29) was significantly shorter compared with normal lines (all greater than 25 passages; n = 6) (Table 1).

DISCUSSION

DM1 LBCLs showed CTG repeat instability with a bias toward expansion in culture. We attributed the gradual increase of the repeat size over several passages to the step-wise mutation (Fig. 1) is consistent with the in vivo model based on the data in PBLs of DM1 patients (Fig. 2A) (8); however, heterogeneity of the size of expanded CTG repeats did not dramatically increase over time, giving rise to the pattern of ‘synchronized’ expansion in most DM1 LBCLs (Fig. 1). There are several potential explanations for this difference. Firstly, the cells studied here are clonal, whereas PBLs in vivo are a mixed group of different cell types with heterogeneous sizes of the expanded CTG repeat alleles. Secondly, our data were obtained within a few months, whereas the duration of the observation was 2–5 years in the in vivo studies. Thirdly, the LBCLs have been transformed with EBV, which may have influenced the stability of the CTG repeat. Fourth, differences between the culture environment and the in vivo environment may have altered the repeat stability.

The majority of gross mutants showed contraction of the expanded CTG repeats as described previously (10). The gross mutants, including relatively rare gross mutants with further expansion, mostly disappeared in the following passages. However, a gross mutant with a further expanded allele occasionally survived to become the modal population of the expanded alleles in later passages (Fig. 3). We have not observed contraction gross mutants surviving more than a few passages. We postulate that this unexpected behavior is due to a growth advantage of the mutant that has a larger CTG repeat expansion over the progenitor cell population. The results of our cell line mixture experiments (Fig. 5) and the correlation between the CTG repeat size and the cell proliferation rate (Fig. 6) strongly support this hypothesis. In a cell culture system undergoing multiple passages, mutant cells would be subjected to genetic drift and must go through a reduction of the population size at each passage. A cell population that has a relatively high proliferation rate has a higher probability for increasing its population size in each passage, although a higher proliferation rate does not always assure survival through passages. It is possible that the same principle has operated in the step-wise mutation, partially accounting for the ‘synchronized’ expansion bias.

Both progenitor and mutant cells share the clonal origin. Thus, all cell populations should have the same genetic background except for mutations that might have arisen after the cloning event. Furthermore, the progenitor and mutant cells
were grown in the same culture flask; therefore, environmental factors were identical. Thus, the putative growth advantage observed in some gross mutants may be attributable to either of two scenarios: (i) a new mutation(s) that arose somewhere in the genome after cloning, or (ii) the CTG repeat expansion itself. The experimental system we used does not allow us to directly determine which of these two possibilities is correct. However, if scenario (i) were the case, there would be two possibilities. One is that such mutations occur preferentially in cells with the repeat size larger than that of the progenitor, and this would suggest that the CTG repeat expansion regulates the mutability of other genes. The other is that an increased cell cycling by a mutation elsewhere confers an increased chance of repeat expansion mutations, implying that faster cell replication promotes expansion mutations. The latter explanation is compatible with models for an expansion bias of the CTG repeat instability based on unusual structures formed by the repeat involving the Okazaki fragment at the replication fork (13,14).

If the larger expansion of the repeat promotes cell proliferation [scenario (ii)], it may have implications for the disease-causing mechanism in DM1. Delayed differentiation has been documented in muscle and brain tissues of congenital DM1 patients (15,16) and in myocyte culture models of DM1 (17,18). It is tempting to speculate the mechanism of these observations in the context of cell proliferation/differentiation coupling, especially the p21WAF1-cyclin-dependent kinases (Cdks)-retinoblastoma tumor suppressor (Rb) network. Down-regulation of p21WAF1 increases cell proliferation, prevents differentiation (19–21) and increases apoptosis (22). Indeed, we demonstrated that the size of CTG repeat expansion shows inverse correlations with cell doubling time and the level of p21WAF1 and a positive correlation with Erk1,2 activities (Fig. 7A), yet the size of the CTG repeat expansion shows correlation with neither Akt activity nor Erk1,2, p53, Bcl-2 or Bax levels in DM1 LBCL subclones. Furthermore, treating the DM1 LBCLs with antisense oligonucleotide upregulated the activation of Erk1,2 (Fig. 8). These observations might suggest that the expansion of the CTG repeat activates Erk1,2 by downregulating the p21WAF1 level, leading to increased cell proliferation. If mutations elsewhere were activating cell cycling, it would be difficult to explain the correlation between the expansion size and specific changes of Erk1,2 and p21WAF1, since such mutations are expected to involve variable pathways to activate cell cycling. Furthermore, our prelim-
inary study suggested that apoptosis is increased in DM1 LBCLs compared with normal LBCLs (data not shown). If this is confirmed, p21WAF1 upregulation might induce apoptosis by a mechanism independent of Bcl-2, Bax and Akt, leading to the shortened lifespan of DM1 LBCLs demonstrated in this study. Further studies are warranted to determine the mechanism involved in the correlations between CTG repeat length, cell cycle signal transduction and apoptosis.

An important question is how relevant our observations in LBCLs are to the situations of DM1 patients in vivo. The gross mutations have not been reported in PBLs of DM1 patients. This may be attributable to the artificial environment of our culture system and the transformation of the cells, which we have already discussed earlier in this paper. However, large intergenerational contractions of the expanded CTG repeat allele to the normal range have been reported (23–25) and mutations with large contractions toward the normal range have been frequently observed in sperm of DM1 patients (8). Mosaicism consisting of two expanded CTG repeat alleles has been reported in the brain of DM1 patients (26). Furthermore, we have recently encountered two patients with the congenital form of DM1 who showed a bimodal distribution of the expanded alleles resembling the bimodal transition phase of the gross mutation seen in our LBCLs (Fig. 4A and B). Furthermore, we have also found similar cases in transgenic mice (27,28). Although some of these observations could also be explained by early embryonic repeat size mutations (29) or intratissue heterogeneity of cell populations, we hypothesize that both frequent step-wise mutations and rare gross mutations do contribute to the in vivo instability of the expanded CTG repeat in patients with DM1. Further investigations of the bimodal alleles of our patients by examining other tissues and follow-up blood samples would be of interest. Since LBCLs are transformed cells derived from an apparently unaffected tissue (i.e. blood) of DM1 patients, we are currently extending our investigations to primary cultures of DM1 muscle cells.

Finally, our data suggest that the association of faster cell proliferation and larger CTG repeat expansion can contribute to the bias of CTG repeat instability toward further expansion; an expansion bias is simply generated by preferential growth of cells with larger alleles over the smaller ones. We have designated this phenomenon ‘mitotic drive’ because it resembles the meiotic drive (segregation distortion) characterized by preferential transmissions of a larger CTG repeat allele from parent to offspring reported in DM1 (30); in either situation, larger CTG repeats are preferentially passed on to the next generation of cells or individuals. Although there have been conflicting data on meiotic drive in DM1 (30,31), meiotic drive is well documented as a biological phenomenon in other species (32–34). ‘Mitotic drive’ may be unique to the DM1 CTG repeat and may not be found with other expanded trinucleotide repeats. Conventional explanations for the expansion bias of the CTG repeat have been based on models involving unusual DNA structure within the Okazaki fragment that consists of a CTG repeat tract (13,14). The ‘mitotic drive’ is a mechanism of expansion bias that is independent of these models, although they are not mutually exclusive. In the post-mitotic cells, such as muscle cells, the ‘mitotic drive’ would only be relevant to their stem cell population and their progeny that are capable of cell divisions. However, DM1 muscles show increased proliferation of myogenic stem cells (i.e. satellite cells) to compensate for muscle loss (16). Thus, ‘mitotic drive’ may be an important mechanism of repeat instability in various tissues of DM1 patients in vivo.

MATERIALS AND METHODS

Patients

Under a consent procedure approved by the local IRB, blood samples were drawn from patients who had both the clinical diagnosis of DM1 and expanded DM1 CTG repeats, and from six normal control subjects (Table 1).

Cloning and passages of LBCLs

PBLs were isolated from blood samples obtained from the 18 DM1 patients using the Ficoll–Hypaque gradient and transformed into LBCL using EBV. The LBCLs were cultured in RPMI-1640 containing 10% heat-inactivated fetal bovine serum and antimycotics (Gibco BRL) in 5% CO2 at 37°C in a 75 cm2 flask (10). Using a hemocytometer, each cell line was single-cell cloned by limiting dilutions at the concentration of 0.5 cells per well of a 96-well plate. The single cell origin of each cell line was ascertained by previous described (10). Each clone was transferred to a 25 cm2 flask once the cell number reached ~1 × 106 cells. Approximately 300 pg of DNA extracted from each clone was analyzed by PCR to confirm the homogeneity of the expanded CTG repeat allele. Cell lines showing two or more major alleles (i.e. multimodal allele size distribution) at this stage were excluded from the study. Clones were then transferred to 75 cm2 flasks and allowed to grow until the cell number reached 3 × 107 cells. Viability of cells was assessed by tripan blue exclusion. An aliquot was harvested for DNA analyses and another aliquot of 1 × 107 cells was used for the Southern blot analysis. The remaining cells were frozen in the culture medium containing 10% DMSO. This passage step was repeated as long as the cells were viable.

SP-PCR analysis

The genomic DNA was extracted using Wizard Genomic DNA Purification Kit (Promega) at each passage. In typical experiments, after amplifiable genome equivalence (a.g.e.) of input DNA was determined by SP-PCR using 3–6 pg of DNA, 20–200 a.g.e. (~60–600 pg) of DNA was analyzed by SP-PCR using primers flanking the DM1 CTG repeat, DMA and DMBR, under PCR conditions previously described (8). The SP-PCR products were subjected to 1.8% agarose gel electrophoresis. After Southern blotting to a nylon membrane (Hybond N; Gibco BRL), the products were hybridized with a32P-γ-ATP end labeled (CAG)10 oligonucleotide probe in 5× SSPE with 0.5% SDS at 42°C overnight.

Southern blot analysis

Southern blot analysis was performed as described elsewhere (1,2).

Mixture experiment

Subclones derived from the same primary clone carrying different sizes of the expanded CTG repeat with an identical genomic background were mixed in pairs (n = 6) in culture. An
Oligos were incorporated into liposomes as described by Tari

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