Implication of Protein Kinase C in the Regulation of DNA Mismatch Repair Protein Expression and Function*

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The DNA mismatch repair (MMR) proteins are essential for the maintenance of genomic stability of human cells. Compared with hereditary or even sporadic carcinomas, MMR gene mutations are very uncommon in leukemia. However, genetic instability, attested by either loss of heterozygosity or microsatellite instability, has been extensively documented in chronic or acute malignant myeloid disorders. This observation suggests that in leukemia some internal or external signals may interfere with MMR protein expression and/or function. We investigated the effects of protein kinase C (PKC) stimulation by 12-O-tetradecanoylphorbol-13-acetate (TPA) on MMR protein expression and activity in human myeloid leukemia cell lines. First, we show here that unstimulated U937 cells displayed low level of PKC activity as well as MMR protein expression and activity compared with a panel of myeloid cell lines. Second, treatment of U937 cells with TPA significantly increased (3–5-fold) hMSH2 expression and, to a lesser extent, hMSH6 and hPMS2 expression, correlated to a restoration of MMR function. In addition, diacylglycerol, a physiological PKC agonist, induced a significant increase in hMSH2 expression, whereas chelerythrine or calphostin C, two PKC inhibitors, significantly decreased TPA-induced hMSH2 expression. Reciprocally, treatment of HEL and KG1a cells that exhibited a high level of PKC expression, with chelerythrine significantly decreased hMSH2 and hMSH6 expression. Moreover, the alteration of MMR protein expression paralleled the difference in microsatellite instability and cell sensitivity to 6-thioguanine. Our results suggest that PKC could play a role in regulating MMR protein expression and function in some myeloid leukemia cells.

DNA mismatch repair (MMR) plays an important role in the maintenance of genomic integrity, as it corrects replicative mismatches that escape DNA polymerase proofreading. Biochemical and genetic studies in eukaryotes have defined at least five genes, hMSH2, hMSH6, hMSH3, hMLH1, and hPMS2, the products of which are required for the human mismatch repair (reviewed in Refs. 1–3). The hMSH2 protein, in combination with either hMSH6 (in a complex called hMutScu) or hMSH3 (hMutSβ complex), binds to the mismatch. Each complex preferentially recognizes a different subset of mismatches. The protein complex hMutScu binds to the single mispairs and small loops, whereas hMutSβ is directed toward the larger loops. Subsequently the recognition complex recruits another heterodimer, hMutLα, comprising hMLH1 and hPMS2, to facilitate mismatch correction.

MMR-deficient cells exhibit a high mutation rate in both coding and noncoding microsatellite sequences (4). In the case of solid tumors, MMR dysfunction accounts for inherited familial cancer syndrome of hereditary non-polyposis colon cancers, and for certain sporadic tumors, including colorectal, endometrial, ovarian, pancreatic, and prostate cancer (5–11). In addition, loss of MMR has been involved in the resistance to DNA damaging agents (reviewed in Ref. 12). Several lines of evidence support the model of toxicity based on abortive attempts to repair DNA damage induced by cytotoxic drugs. Consequently, the loss of MMR activity contributes to cell resistance to methylating agents (13) and 6-thioguanine (6-TG) (14), as well as a few other compounds (12, 15–17). Different mechanisms are involved in the MMR inactivation process related to the development of cancer: (i) a first mutation, either germinal (hereditary cancer) or somatic (sporadic cancer), followed by another somatic event (18); (ii) an epigenetic silencing mechanism such as the loss of hMLH1 expression associated with promoter methylation of the hMLH1 gene (19, 20); (iii) a deregulation of the hMSH3 expression such as overexpression of hMSH3 by gene amplification that sequesters hMSH2 into the hMutSβ complex, resulting in hMutScu activity deficiency (21, 22).

In the case of leukemia, genetic instability with loss of heterozygosity or microsatellite instability (MSI) has been observed in acute leukemia, myelodysplasia, and chronic myeloid leukemia cells (23–29). On the other hand, MSH2 knock-out mice have an increased propensity to develop lymphoma (30).

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†† The abbreviations used are: MMR, mismatch repair; AML, acute myeloid leukemia; DAG, diacylglycerol; DiC8, 1,2-dioctanoyl-sn-glycerol-3-phosphate; EMSA, electrophoresis mobility shift assay; FCS, fetal calf serum; MSI, microsatellite instability; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MBF, myelin basic protein; PKC, protein kinase C; 6-TG, 6-thioguanine; TPA, 12-O-tetradecanoylphorbol-13-acetate.
Involvement of PKC in Mismatch Repair Activity

However, MSI is quite infrequent in primitive myeloid leukemia (31, 32), some reports suggesting its occurrence in secondary and therapy-induced leukemia (33, 34) or the opposite (35). Investigations on the presence of mutations in MMR genes have shown that these mutations are uncommon events in leukemia (36). Thus, these observations show that MMR deficiency is rarely involved in leukemia cell genetic instability. However, it is conceivable that, in leukemia cells, MMR deficiency does occur as a result of negative transcriptional or post-transcriptional regulatory mechanisms. Little is known about the intracellular or extracellular signals that may influence MMR gene expression. A recent report pointed out that MSI was found in acute myeloid leukemia (AML) with abnormal expression of hMSH2 protein in a significant proportion of the patients (33).

Therefore, to obtain new insights into a putative control of MMR by internal or external signals, we evaluated the influence of phorbol ester-induced protein kinase C (PKC) stimulation on MMR protein expression and function in monocytic acute leukemia U937 cells. PKC, a serine-threonine kinase (for review, see Ref. 37), plays a pivotal role in signal transduction, and its activation was reported to regulate the methylguanine methyltransferase (MGMT) DNA repair gene (38, 39). Thus, the potential involvement of PKC activation on the expression of MMR protein should be considered.

In this study we present evidence that MMR protein expression was induced in U937 cells when treated with phorbol ester that stimulates PKC activity (39). Reciprocally, PKC inhibition led to a decrease of MMR protein expression. Variations of protein expression correlated with the MMR activity as determined by in vitro assays and cell response to the cytotoxic agent, 6-TG. These results suggest a direct and/or indirect involvement of PKC in the regulation of MMR protein expression and function in myeloid cells.

MATERIALS AND METHODS

Cell Lines and Chemicals—HeLa cells were from the European Molecular Biology Laboratory (Heidelberg, Germany). Six AML cell lines derived from distinct stages of myeloid differentiation were used, and their immunophenotypes have been previously reported (40). U937 (monocytic) (41), HL-60 (myelocytic), HEL (erythromyeloblastic), KG1a (pre-myeloblastic), and Jurkat (lymphoid) cell lines were obtained from the American Type Culture Collection (Rockville, MD). MMR by internal or external signals, we evaluated the influence of phorbol ester-induced protein kinase C (PKC) stimulation on MMR protein expression and function in monocytic acute leukemia U937 cells. PKC, a serine-threonine kinase (for review, see Ref. 37), plays a pivotal role in signal transduction, and its activation was reported to regulate the methylguanine methyltransferase (MGMT) DNA repair gene (38, 39). Thus, the potential involvement of PKC activation on the expression of MMR protein should be considered.

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Determination of PKC Activity—PKC activity was determined in cell extracts as previously reported (43). Briefly, total PKC was determined by measuring the incorporation of ^3^P into myelin basic protein (MBP). Cells (5 × 10^7^) were lysed in 20 mM Tris-HCl, pH 7.4, 60 mM glycerophosphoric acid, 10 mM EGTA, 20 mM MgCl_2, 0.1 mM sodium fluoride, 2 mM dithiothreitol, 1 mM sodium orthovanadate, 20 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, for 15 min. The assay was initiated by the addition of 0.1 mM ATP, 2.5 μM/ml MBP, and [γ-^3^P]ATP (3000 Ci/mmol). After 30 min at 30 °C, the reaction was stopped by the addition of 10% trichloroacetic acid and collected on Whatman GF/C filters. Following extensive washes with 10% trichloroacetic acid, washes, and mounted with an anti-fading solution (Vector Laboratories). Slides were examined under a confocal laser scanning microscope with a 63× oil immersion objective. The confocal imaging system was a Zeiss (Oberkochen, Germany) scanning assembly incorporating argon ion laser (568 nm), a 543 nm He-Ne laser, and a Zeiss Plan APO 100×, 1.4 NA oil immersion objective. Images were digitized and photographs were taken using ILford FP4 films (44). Laser settings were kept identical for the examination of samples to be compared.

Mismatch Binding Assay—The substrates were 34-mer duplex ^3^P-labeled oligonucleotides containing a single G-T mispair (45) or a matched G-C as a control. Band shift assays were performed as previously reported (46) except for the use of replicative cell extracts (150 μg). Briefly, extracts were incubated for 5 min at room temperature with 2 pmol of non-radioactive competitor duplex in 15 μl of reaction buffer (25 mM Hepes-KOH, pH 8.0, 0.5 mM EDTA, 0.5 mM dithiothreitol, 10% glycerol). The radiolabeled duplex oligonucleotide (20 fmol) was then added and incubation continued for another 20 min. Reactions were analyzed by electrophoresis on 6% polyacrylamide gels, and the products were detected by autoradiography.

Mismatch Repair Assay—Repliecat cell extracts used in the in vitro assay were prepared as described (47). Substrates for mismatch correction were nicked circular molecules containing a single TC mispair constructed as described (48). Repair assays were carried out as previously reported (48). Briefly, correction of the TC mispair restored a Mlu I restriction site. The heteroduplex substrate (90 ng) with cell extracts (up to 200 μg) for 60 min at 37 °C in a buffer (25 μl) containing 30 mM Hepes-KOH, pH 8.0, 7 mM MgCl_2, 0.5 mM dithiothreitol, 0.1 mM each dNTP, 4 mM ATP, 40 mM phosphocreatine, 1 μg creatine phosphokinase, and DNA purification, repair was checked by restriction enzyme digestion followed by gel electrophoresis. Specific repair of the mismatch heteroduplex by proficient extracts resulted in the generation of a new Mlu I site, whereas non-correction by deficient extracts led to a simple linearization of the substrate (4.5 kb).

Determination of Cell Survival after Genotoxic Treatment—The toxicity of 6-TG was evaluated by the MTT assay, performed as described elsewhere (49). Briefly, 2 × 10^5^ cells/well were plated, then treated with the drug. After a 72-h incubation time period, cells were centrifuged and the MTT assay was performed. The survival curves were determined with the solvent (water) alone as a control, and the percentages of viable cells were determined by spectrophotometric measurement. Drug concentrations that correspond to 50% of viable cells (IC_{50}) were determined and used to compare cell sensitivities to 6-TG treatment.

DNA Extraction and MSI Analysis—MSI was assessed in U937 (four clones), HL-60 (two clones), KG1a (two clones), and HEL (one clone) cell lines and compared with Jurkat cell line. Genomic DNA was extracted from cells using a DNA extraction kit (Amersham Biosciences) following the manufacturer’s instructions. DNA from leukemic cell lines was investigated using two microsatellite markers (BAT25 (intron 16 of c-kit oncogene) and BAT26 (intron 5 of hMSH2) for MSI. Primers were synthesized by MWG-Biotech (France) and were fluorescently labeled. PCR reaction was set up in a 50 μl volume containing 2 μl of DNA, 25 μM amounts of each primer, 2.5 μM MgCl_2, 0.5 mM dNTP, and 1.25 units of Taq Polymerase (PerkinElmer). Amplification was performed in a model 480 thermocycler (PerkinElmer) after an initial denaturation at 94 °C for 10 min, followed by 28 cycles of denaturation at 94 °C for 1 min, hybridization at 50 °C for 1 min, and polymerization at 72 °C for 1 min, and a final extension step at 72 °C for 10 min. Fluorescently labeled PCR products were detected using an automated
Significant.

Levels of expression of hMSH2, hMSH6, hMLH1, and hPMS2 in U937 times of the chemiluminescent signals were used to detect weaker was used as a loading control (data not shown).

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GeneScan and Genotyper software for automatic sizing of fragments. ABI 377 DNA sequencer. Data analyses were performed using ABI Genescan and Genotyper software for automatic sizing of fragments.

Statistical Analysis—Statistical significance was calculated using Student's t test. A probability value < 0.05 was considered to be significant.

RESULTS

Phorbol Ester TPA Increases MMR Protein Expression in U937 Cells—Whole cell extracts of HeLa (used as control) and leukemia cell lines were prepared from cells in exponential growth phase. In HeLa cells the expected protein bands of 105, 160, 85, and 115 kDa for hMSH2, hMSH6, hMLH1, and hPMS2, respectively, were detected by Western blotting (Fig. 1A). The expression of MMR proteins varied among the different cell lines used. The expression of hMSH2 in HEL cells was similar to that of HeLa cells, whereas it was decreased in U937 cell extracts and was detected only after prolonged chemiluminescence exposure (Fig. 1B). hMSH2 expression level was 8-fold lower in U937 cells than in HeLa and HEL cells (Fig. 1C). Because hMSH2 belongs to a protein complex, the expression of the other partners that participate to the MMR reaction was investigated in these cell lines. We found that the levels of hMSH6 and hPMS2 were also decreased in U937 cells compared with HEL and HeLa cells, whereas hMLH1 expression in U937 cells was comparable with that in HeLa cells (Fig. 1, A–C).

We next evaluated the effect of TPA, a PKC activator, in the U937 cell. Treatment with TPA (50–100 nM) of U937 cells resulted in a 5-fold increase of hMSH2 expression after a 24-h time period (Fig. 1C). The kinetic of TPA effect showed an increase detectable after 1 h of incubation, further increasing by 3-fold after 3 h and reaching a plateau at 24 h that lasted up to 72 h (Fig. 1D and data not shown). The overexpression of hMSH2 was accompanied by an increase in hMSH6 expression (Fig. 1C). TPA treatment also increased the expression of hPMS2, albeit at a lesser extent (Fig. 1C).

Phorbol Ester TPA Increases MMR Protein Expression through PKC Stimulation—First, to check whether the effect of TPA in U937 cells was not cell line specific, we examined hMSH2 protein expression in various AML-derived cell lines. As shown in Fig. 2A, the levels of hMSH2 analyzed by Western blotting were in the same range in HEL, KG1a, UT-7, TF-1, and HeLa cells but were significantly lower in U937 and HL-60 cells. As expected, no hMSH2 expression was found in Jurkat cells that are defective in hMuta (50). The overexpression of hMSH2 observed with U937 cells after TPA treatment was also found in HL-60 cells (Fig. 2B). Interestingly, treatment of U937 and HL-60 cells with 50 nM TPA resulted in a 3–4-fold PKC stimulation that lasted for several hours (Ref. 43 and Table 1).

Second, we investigated whether TPA treatment could increase hMSH2 expression in cells displaying high basal level of expression. In contrast to U937 and HL-60 cells, the high levels of hMSH2 expression in HeLa and HEL cell lines were not modified after TPA treatment (Fig. 2B). However, KG1a and TF-1 cells, which display a higher level of hMSH2 expression than U937 and HL-60 cells, also exhibited a 2–5-fold higher basal PKC activity compared with these cells (Table 1).

Third, TPA can induce various biological effects in addition to PKC activation; therefore, we investigated whether PKC stimulation could play a role in hMSH2 overexpression. Thus, we evaluated the effect of diacylglycerol (DAG), a physiological although less potent PKC agonist (51). Indeed, a 24-h incubation with DiC8 (25 mg/ml), a cell-permeant DAG analog, increased hMSH2 expression, albeit to a lesser extent than TPA (Fig. 2C). Moreover, the effect on TPA-induced hMSH2 overexpression in U937 cells of calphostin C and chelerythrine, two PKC inhibitors, was investigated. As shown in Fig. 2C, calphostin C (100 nM) or chelerythrine (10 mM) significantly inhibited the effect of TPA on hMSH2 expression. We then examined whether hMSH2 content in cells with high level of expression compared with U937 and HL-60 cells could be decreased by treatment with a PKC inhibitor. After chelerythrine treatment of HEL and KG1a cells during 24 h, the expression of hMSH2 and hMSH6 was significantly decreased (Fig. 2D). Finally, PKC depletion in HEL cells after prolonged exposure (72 h) at high TPA concentration (100 nM) resulted in a 2.5-fold decrease in hMSH2 protein expression (data not shown).

Intracellular Localization of hMSH2—Because protein expression and MMR activity were determined with total cell lysates, the increase of hMSH2 content could have been restricted to cytoplasm and consequently would not be directly responsible for changes in MMR activity. Therefore, we carried out confocal analysis to evaluate the influence of PKC stimulation on hMSH2 localization. In U937 cells treated with 50 nM TPA for 24 h, immunostaining with anti-hMSH2 antibody gave higher fluorescence signals, compared with controls (Fig. 3), as expected from Western blot analysis. Moreover, we found that hMSH2 was mostly localized in the nucleus, whereas a faint punctate fluorescence was also detected in the cytoplasm in both TPA-treated and untreated U937 cells. These results suggest that PKC stimulation did not affect the intracellular localization of the hMSH2 protein, which remained mostly nuclear.

Phorbol Ester TPA Increases MMR Protein Binding—The
FIG. 2. Expression of hMSH2 in various cell lines and effect of activators and inhibitors of PKC. Experimental conditions were identical to those in Fig. 1. A, expression of hMSH2 protein in various cell lines. B, effect of TPA treatment on hMSH2 protein expression in HeLa, U937, HEL, and HL-60 cells. C, expression of hMSH2 protein in U937 cells treated with Dic8 (50 nM). Effect of calphostin C (C.C) (100 nM) and chelerythrine (Chel.) (10 μM) pretreatment on hMSH2 expression in U937 cells treated with TPA. D, effect of chelerythrine (10 μM) on hMSH2 and hMSH6 expression in HEL and KG1a cells. CT, control without treatment.

TABLE I
PKC activity in leukemia cell lines

| Cell line | PKC activity | TP1 | KG1a | U937 | U937 + TPA | HL-60 | HL-60 + TPA |
|-----------|--------------|-----|------|------|------------|-------|------------|
|           |              | 57  | 101  | 27   | 105 ± 10   | 25    | 104 ± 21   |

The whole PKC activity was determined after 1 h of TPA treatment (50 nm) as described under “Materials and Methods.” PKC activity is expressed as pmol of 32P/min/10^6 cells. Each value is the mean of three independent experiments with S.D.

G-T complex was observed with all the substrates and extracts tested, reflecting a binding activity by other recognition factors that interact with duplex DNA in our experimental conditions. Supershift EMSA experiment was used to ascertain the heteroduplex binding activity of extracts of TPA-treated U937 cells. Addition of antibody raised against hMSH6 at the end of the reaction resulted in a supershift retardation mobility band observed both with HeLa and TPA-treated U937 cells and consistent with the formation of hMSH2 and hMSH6 protein complex (Fig. 4B).

Phorbol Ester TPA Increases MMR Activity—In addition to the binding activity, the complete MMR capacity was then verified by an in vitro MMR assay (48, 52). Our standard assay measures the correction of a single TC mispair carried by a nicked circular duplex molecule. The efficiency of the mismatch repair correction was followed by the appearance of a diagnostic band corresponding to the restoration of a MluI restriction site. Extracts of HeLa and HEL cells efficiently corrected the mismatch (Fig. 5A). More than 50% of the substrate was repaired by 100 μg of proteins of both cell extracts. The absence of any diagnostic band after incubation with Jurkat extracts attested for the hMSH2 mutation in these cells. Consistent with their defect in binding capacity, unstimulated U937 cells exhibited an inability to carry out detectable correction under our experimental conditions. Again, TPA favorably influenced the repair activity because treatment of U937 cells significantly increased the repair of the TC substrate to a fully detectable level (25–30% of repair as determined by the intensity of the repair band). The increased activity in TPA-treated U937 cells cannot be quantified by the assay because there was no activity in untreated cells and the assay is essentially qualitative rather than quantitative.

To authenticate the absence of apparent correction by U937 cells, we used two independent batches of cell extracts (Fig. 5B). In both cases, the TC substrate was not repaired to a matched molecule. Fifty μg of TPA-stimulated cell extracts were already sufficient to firmly observe a repair band on a gel, whereas up to 200 μg of nontreated cells did not lead to any significant repair. Moreover, mixing the inactive U937 cell extracts with active HeLa cell extracts did not affect the level of
Assay. Two independent extract preparations are shown for U937 cells.

Deficient Jurkat cell line exhibit resistance to 6-TG (IC50).

A range of 50-200 μg of each extract was used in the in vitro repair assay. Two independent extract preparations are shown for U937 cells.

correction of the TC substrate (data not shown), thus excluding the presence of an inhibitory activity in the U937 extracts.

Phenotypic Features Associated with Repair Defect—MMR activity in cells can be evaluated either directly or indirectly. Besides the biochemical evidences given by the in vitro assays, the latter approach may be based on the cell response to 6-TG, as expected, the MMR-deficient Jurkat cell line exhibit resistance to 6-TG (IC50 = 2.85 ± 0.43 μM), similar to that observed with HL-60 and U937 cells (IC50 = 2.38 ± 0.10 μM and 2.27 ± 0.11 μM, respectively). To further confirm functional loss of MMR in U937 and HL-60 cell lines, we performed MSI analysis. Mononucleotide microsatellites have been shown to be particularly prone to mutations and can be used to assess instability (35). We used two mononucleotide microsatellites, BAT25 and BAT26, tested previously in leukemia (35). Although instability was not observed at BAT26 locus in our leukemic cell lines (excepted Jurkat cell line), MSI was reproducibly detected at BAT25 locus, in all clones of U937, HL-60, and Jurkat cell lines (Fig. 7). These results support the findings that U937 and HL-60 cell lines exhibit a deficit in both expression and activity of MMR components.

DISCUSSION

The human MMR system may be differently regulated in a number of biological situations. Our work focused on the effect of PKC stimulation on the expression and function of the MMR proteins in leukemia cells. We showed that, among myeloid cell lines, monocytic U937 cells naturally exhibited a low level of repair proteins, correlated in vitro to a down-regulation of activity. In addition, we provided evidence that the stimulation of PKC, by either TPA or the physiological agonist DAG, resulted in an increase of MMR protein expression with subsequent increase of MMR protein function. Conversely, this induction was inhibited by PKC antagonists. Similarly, we extended these findings to the HL-60 cells.

Little is known about the level of MMR protein expression in leukemia cells. Our data reveal that hMSH2 and other MMR protein expression levels may differ from one myeloid cell population (KG1a, HEL, UT-7, TF1) to another (HL-60 and U937). KG1a, HEL, UT-7, and TF1 cells express the early differentiation marker CD34, whereas U937 and HL-60 cells do not, suggesting the existence of a correlation between hMSH2 expression and early differentiation status. This observation is in agreement with other studies reporting a predominant expression of hMSH2 located in the proliferative compartments of the esophageal and intestinal epithelia (54, 55). The mechanism that accounts for these differences remains uncertain. However, based on the potential role of PKC in MMR expression and function, it is interesting to note that the higher basal PKC activity in KG1a, HEL, UT-7, and TF1 cells compared with
U937 and HL-60 cells paralleled the level of MMR protein expression (Table I and Fig. 2A). Therefore, it is possible that differences in basal PKC activity contribute to the differences in MMR capacity.

We found that TPA- or DAG-induced PKC stimulation in U937 cells results in amplification of the cellular content in MMR proteins. As it has been extensively documented elsewhere, prolonged exposure to TPA induced terminal monocytic differentiation of U937 and HL-60 cells attested by cell adherence, monocytic morphological features, and increase of CD14 expression. However, our study suggests that MMR protein overexpression could not be necessarily coupled to terminal differentiation. Indeed, MMR protein overexpression is an early event that largely anticipated differentiation features. Furthermore, DiC8, a much less potent differentiating agent than TPA (56), and one that is unable to induce cell terminal U937 monocytic differentiation (data not shown), was able to up-regulate MMR protein expression. In human cell lines, the level of hMSH2 protein changes during cell cycle or the differentiation process. It has been reported that hMSH2 was expressed at a basal level in resting cells but induced in proliferative phase (57). In this respect, as TPA treatment induces differentiation, proliferative phase could not account for up-regulation of hMSH2.

The increase of MMR protein levels in total cell extracts could have been associated with a redistribution mechanism of the hMSH2, i.e. nuclear translocation. Interestingly, the increase in nuclear hMSH2 level after treatment with monoalkylating agents was reported to be caused by translocation from the cytoplasm to the nucleus compartment (58). However, confocal analysis allowed us to rule out such a redistribution mechanism after TPA treatment of U937 cells.

The levels of MMR proteins detected in cell extracts accurately correlated with repair capacity, as attested by biochemical evidence obtained with in vitro assays. They have been also
correlated with phenotypic characteristics. Indeed, we showed that the low MMR expression conferred a resistant phenotype to 6-TG treatment in U937 and HL-60 cells compared with HEL and KG1a cells. The major contribution of MMR to the cytotoxic effects of this drug and the emergence of resistance phenotype resulting from loss of MMR activity have been described in details (for review, see Refs. 3 and 12). Furthermore, U937 and HL-60 cells exhibited MSI at the Bat25 locus (Fig. 7). However, because MMR expression was decreased but not abolished in U937 and HL-60 cells, the MSI+ phenotype, hallmark of MMR deficiencies, might be attenuated in these cells compared with the hMSH2−/− Jurkat control. Additionally, it has been reported that significant MSI is not exhibited by all cells proven to be deficient in MMR activity by biochemical assays (59).

The reversible deficit of MMR proteins adjusted by TPA treatment is correlated to a restoration of MMR function. Up-regulation of protein expression might be because of either a direct or indirect mechanism. A direct effect could be associated with a transcriptional regulation. Gene activation mediated by PKC through activation of AP-1 transcription factor has been described for MGMT and ERCC1 repair genes (38, 60). AP-1 transcription factors are activated by TPA incubation (61). The possibility of transcriptional regulation of MMR genes by AP-1 transcription via PKC stimulation is currently examined to elucidate a functional role of the AP-1 sequence within the hMSH2 promoter. Notably, upon UV irradiation, the transcription of hMSH2 is up-regulated and critically depends on functional interaction with c-Jun (62). In a preliminary experiment, we observed a time-dependent increase in hMSH2 mRNA following treatment of U937 cells with TPA (data not shown). Nevertheless, an indirect effect such as the reversal of protein expression inhibition by a PKC-dependent mechanism cannot be ruled out and PKCs could directly influence MMR function through another mechanism. One hypothesis is that PKC could modulate the serine/threonine phosphorylation status of the MMR proteins because hMSH2 protein contains five putative PKC phosphorylation sites. In this perspective, it is interesting to note that some PKCs have been found to be associated with the nucleus either constitutively or after translocation upon stimulation (63). At last, PKCs are divided into three groups (conventional, novel, and atypical) based on structural differences (37). As we determined the global PKC activity, the nature of the PKC isoform(s) implicated in the regulation of MMR protein expression could not be determined but is under investigation.

Quantitative changes observed in the cellular content of the MMR components are not equal for all of these proteins (Fig. 1). The effect is relatively attenuated in the case of hMLH1, whereas it is markedly more pronounced for hMSH2. Actually, U937 cells contain scarcely detectable level of hMSH2. Even if it is not a total loss of the protein, this may be sufficient to completely inhibit the repair reaction, just as diminished hMLH1 gene expression is associated with cancer susceptibility (19). Absolute cellular amounts of every component of the human MMR may be less important than their relative ratios for a functional repair system as exemplified recently (64). Moreover, hMSH2 plays a role in stabilizing hMSH6 that disappears in the absence of hMSH2 (65). Thus, the faint hMSH2 level would be sufficient to explain both a weak hMSH6 level and a repair deficiency.

In summary, our results indicate that PKC activity contributes to both MMR protein expression and function in leukemia cells. We found that low levels of PKC activity corresponded to low levels of MMR expression and that, upon activation of PKC with TPA, the expression of MMR proteins was increased. Therefore, it is possible that any internal or external signals leading to DAG production and subsequent PKC stimulation may greatly enhance MMR capacity. Conversely, it is conceivable that reduced expression or function of some critical PKC isoform(s) may result in MMR deficiency, with important consequences in terms of mutagenesis and drug function. Therefore, we are grateful to Dr. J. P. Jaffrézic and Dr. G. Villani for critical reading of the manuscript.

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