# PML and TRF2 protein expression in hereditary and sporadic colon cancer

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The PML *(*promyelocytic leukemia) protein is concentrated in the PML nuclear bodies. In human cell lines and tumors maintaining their telomeres by alternative lengthening (ALT), the PML protein is colocalized with TRF2 and several other proteins in the so called ALT-associated PML bodies. The aim of this study was to determine if there is any difference in PML protein expression between tumors with stable microsatellites (MSS) and those with high-frequency microsatellite instability (MSI-H), if PML protein expression might be a prognostic factor and if MSI-H tumors more frequently use alternative lengthening of telomeres measured by the presence of ALT-associated PML bodies. Eighty colorectal cancer samples (32 MSI-H and 48 MSS) and 8 human tumor cell lines (Saos-2, U2OS, DU145, LNCaP, U87, HeLa, MCF7 and T98G) were included into the study. Double-colour immunofluorescence staining was used. Downregulation of PML protein expression was found in 7 of 32 (22%) MSI-H and 11 of 48 (23%) MSS tumors (p=0.520). There was no correlation between PML expression and age, histological typing, localization of the tumor in colon, TNM classification, disease-free and overall survival. The Saos-2 and U2OS (ALT using cell lines) and the MCF7 (active telomerase) cell line were characterized by the presence of ALT-associated PML bodies; no such bodies were detected in the DU145, LNCaP, U87, HeLa and T98G cell lines (active telomerase); accumulation of TRF2 was absent or much weaker in these cell lines compared to Saos-2 or U2OS. Accumulation of the TRF2 protein was detected in 16 of 80 (20%) tumors and PML and TRF2 colocalization in 2 MSI-H tumors (6%). In conclusion, the PML protein was downregulated in approximately 20% of tumors; there was no difference between MSS and MSI-H tumors. PML protein expression does not seem to be a prognostic factor.

*Key words: PML; TRF2; alternative lengthening of telomeres; hMLH1 – gene; hMSH2 – gene; microsatellite instability.*

*PML (*promyelocytic leukemia) is a tumor suppressor gene that encodes a multifuntional protein with critical tumor suppressive functions such as induction of apoptosis, growth arrest and cellular senescence [1]. The PML protein has been shown to be concentrated in subnuclear structures named PML nuclear bodies, having a role in their formation and stability [2]. PML bodies represent nuclear deposits of various proteins [3]. To date, more than 50 proteins have been found to colocalize with PML in the bodies either transiently or constitutively [2, 4]. Based on interactions of these proteins and the apparently regulated recruitment/release of proteins upon some signaling pathways induced by external insults, a regulated depot function has been postulated for PML bodies [5]. Through PML bodies PML harmonically coordinates his tumor suppressive functions including regulation of both p53 – dependent and p53 – independent pathways for apoptosis at the transcription level [1, 5].

The *PML* gene is consistently disrupted in acute promyelocytic leukemia. Due to a chromosome [15;17] translocation, the PML protein forms a fusion protein with retinoid acid receptor α (RAR α). Presence of PML-RAR α fusion protein disrupts PML bodies in acute promyelocytic leukemia cells and treatment with arsenic and retinoic acid restores PML bodies via an unknown mechanism [6-9].

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In the great majority of malignant tumors and immortalized human cells, telomerase activity can be detected. Reactivation of telomerase activity is associated with the possibility to overcome the „end-replication problem" of chromosomes in tumor cells and the process of immortalization [10]. Some immortalized human cell lines and tumors have no detectable telomerase activity and maintain their telomeres by a mechanism known as alternative lengthening of telomeres (ALT) based on homologous recombination and copy switching of telomeric sequences [11, 12]. ALT cell lines and tumors were described to contain a novel form of PML body in which the PML protein colocalizes with telomeric DNA, the telomere binding proteins 1 and 2 (TRF1;TRF2) and several proteins involved in DNA repair and recombination, including replication factor A, RAD51 and RAD52 [13-16]. These structures were not detected in mortal cells or in telomerase-positive cell lines and tumors and are referred as ALT-associated PML bodies, APBs [13, 14]. Colocalization of these molecules in APBs using immunohistochemistry is compactible with these structures being involved in ALT [16]. Defects in the DNA mismatch repair machinery in telomerase-defective budding yeast promote telomerase-independent proliferation, indicating that the antirecombination activity of the MMR machinery might have an inhibitory effect on the ability of telomerase-defective cells to proliferate [17].

About 5% of all cases of colorectal cancer are associated with hereditary non-polyposis colon cancer (HNPCC), a syndrome of cancer predisposition caused by germline mutations in DNA mismatch repair genes including *hMLH1, hMSH2, hMSH6, hPMS2* and *hMLH3* [18-23]. These cases are associated with the phenotype of high–frequency microsatellite instability (MSI-H), as well as approximately 10-15% of sporadic tumors [24, 25]. Tumors with microsatellite instability differ in some characteristics from microsatellite stable (MSS) tumors, including predominant euploidy, rare 18q loss of heterozygosity, rare p53 mutations, low EGFR protein expression, unique pathologic attributes and better prognosis (26-28). There are no reports on PML and TRF2 protein expression in MSI-H colon cancer.

The aim of this study was to determine: 1) if there is any difference in PML protein expression between tumors with and without DNA mismatch repair defect (i.e. with and without microsatellite instability); 2) if PML protein expression might be a prognostic factor; 3) if in accord with the above mentioned yeast studies tumors with DNA mismatch repair defects more frequently use the mechanism of alternative lengthening of telomeres compared to microsatellite stable tumors measured by the presence of ALT-associated PML bodies.

## Materials and methods

*Patients.* Patients with colon cancer were included into the study that were previously analyzed for suspect hereditary nonpolyposis colon cancer. Informed consent was obtained from the patients. Formalin fixed paraffin-embedded tumor samples were studied. There were 80 colorectal cancer samples, 32 MSI-H and 48 MSS ones. Of the 32 MSI-H samples, 15 and 4 were from *hMLH1* and *hMSH2* gene mutation carriers, respectively, 13 were sporadic. From two *hMLH1* gene mutation carriers, a fresh frozen tumor sample was available.

*Cell lines.* In order to optimize the analysis of alternative lengthening of telomeres we analyzed 8 human tumor cell lines with a known status of telomerase activation and presence of ALT mechanism, including Saos-2 (osteosarcoma), U2OS (osteosarcoma), DU145 (prostate), LNCaP (prostate), U87 (glioblastoma-astrocytoma), HeLa (cervical), MCF7 (breast adenocarcinoma) and T98 (glioblastoma) cell lines. Cultivation conditions were following: for Saos-2, U2OS, DU145, U87, HeLa, MCF7, T98 cell lines: DMEM (Dulbeco's modified Eagles Medium; Invitrogen, USA) supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine (Invitrogen, USA), 1 U/ml penicilin (Sigma, USA) and 1 μg/ml streptomycin (Sigma, USA); for LNCaP cell line: RPMI (Roswell Park Memorial Institute) 1640 medium (Sigma, USA) supplemented with 10% FCS, 2 mM L-glutamine (Invitrogen, USA), 1 mM sodium pyruvate (Sigma, USA), 1 U/ml penicilin (Sigma, USA) and 1 μg/ml streptomycin (Sigma, USA). All cell lines were maintained under standard cell culture conditions at 37°C and 5 % $CO_2$ in a humid environment.

*Double-colour immunofluorescence staining* with anti-PML and anti-TRF2 antibodies was performed. Formalin-fixed paraffin-embedded tumor tissue sections 4-μm thick were deparaffinized and heat-induced antigen retrieval was performed before staining by treatment in a microwave oven (30 minutes at 700 W, in 10 mM citrate buffer, pH 6,0).

Cell lines were grown on coverslips in tissue culture dishes and collected. The cells were washed with phosphate buffered saline (PBS), fixed with methanol and acetone (1:1, -20°C for 10 min) and washed in PBS again, being thus prepared for staining.

The following procedures were the same in both formalin-fixed samples and cell lines. Unspecific binding sites were blocked by a solution of fat-free dried milk (2,5 mg of dried milk/50 ml PBS). Samples were incubated with mouse monoclonal antibody against TRF2 (Imgenex, USA, dilution 1:50) at 4°C overnight. The following day, samples were washed in PBS 3 times and secondary fluorescent anti-mouse antibody Alexa-Fluor®594 (Molecular Probes, Oregon, USA) was applied for 1 hour at room temperature. After washing 3 times in PBS, samples were incubated with rabbit polyclonal antibody against PML (clone H-238, Santa Cruz Biotechnology, USA, dilution 1:200) for 2 hours at room temperature. The samples were washed 3 times in PBS and secondary fluorescent anti-rabbit antibody Alexa-Fluor®488 (Molecular Probes, Oregon, USA) was applied for 1 hour at room temperature. The samples were washed in PBS again and DAPI (Sigma, St. Louis, USA) was applied for 10 minutes. After 2 washes in PBS and one in deionized water the samples were mounted

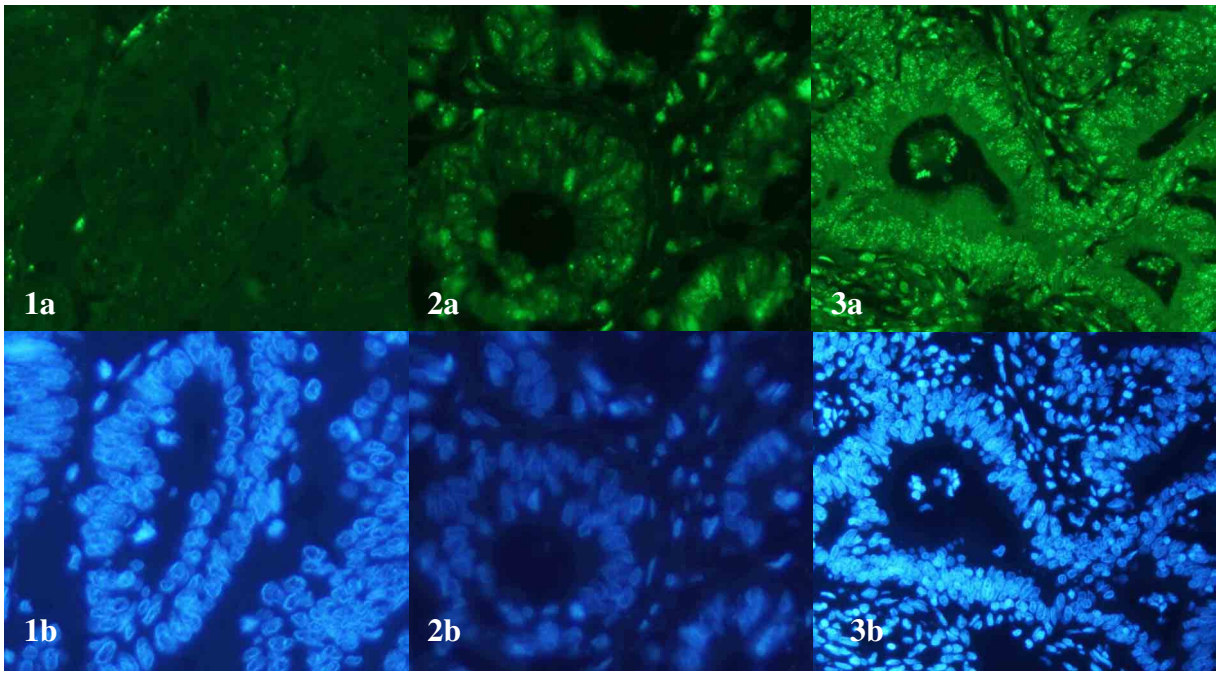


**Figure No. 1. PML protein expression in colon cancer samples (1-3a, PML protein expression: 1a, level "1" positivity; 2a, level "2" positivity; 3a, level "3" positivity; 1-3b, dapi; magnification 200x).**

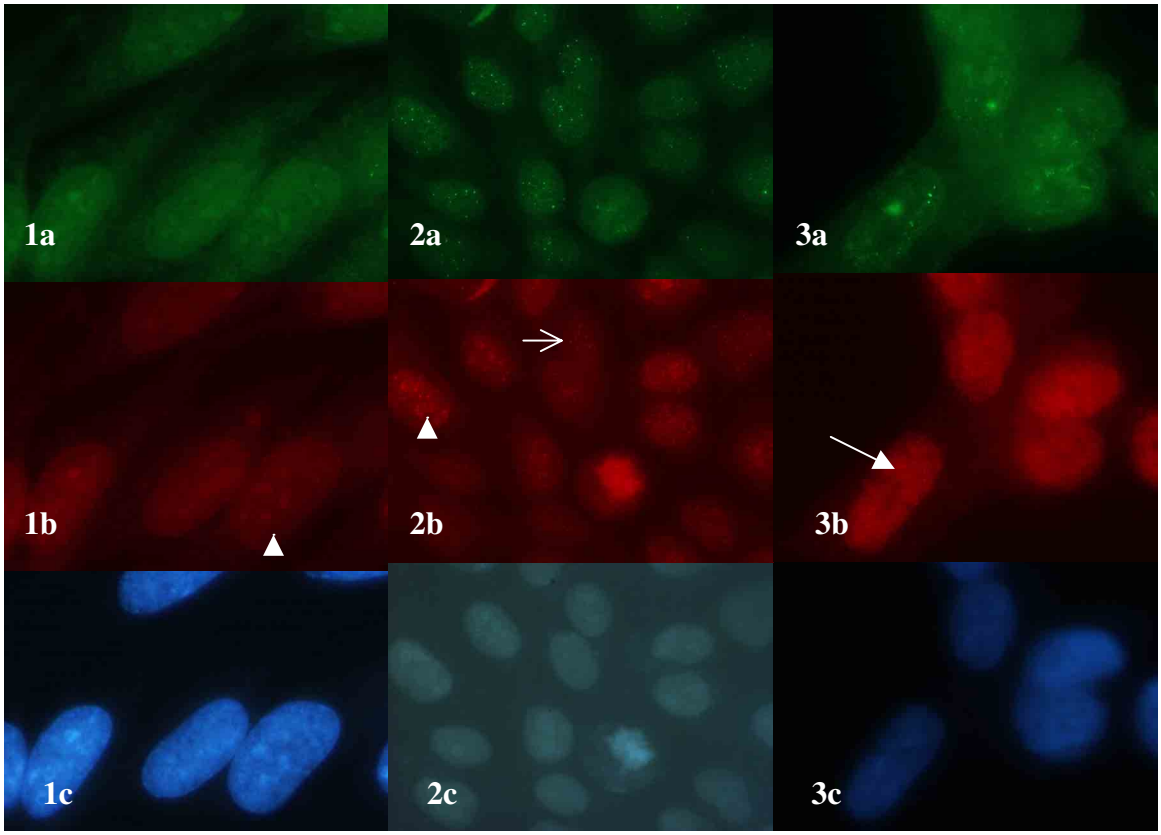


**Figure No 2. PML and TRF2 protein expression in cell lines (1, Saos-2 cell line; 2, DU145 cell line; 3, LNCaP cell line; a, PML protein expression; b, TRF2 protein expression [pattern of TRF2 bodies: large aggregates (▲), small aggregates (➔), diffuse granular pattern (➞)]; c, DAPI; magnification 1000x).**

in water medium. The slides were visualized using an Olympus U-RFL-T fluorescent lamp and an Olympus BX50 microscope at 100x magnification. Images were captured with the Viewfinder imaging system. Captured images were processed using the Adobe Photoshop program.

As regards anti-PML antibody, we first compared the sensitivity of the H-238 antibody with the well characterized PG-M3 antibody (Santa Cruz Biotechnology, USA). We obtained similar results.

The number and size of PML bodies was assessed in the nuclei of the cells, the bodies staining green. Nuclear staining was graded semiquantitatively. Complete absence of PML bodies was scored as "0"; the presence of minute bodies in less than 50% of cells as "1"; the presence of PML bodies of any size in 50-90% of cells or 1-2 minute bodies in 100% of cells as "2"; 1-2 large bodies or 3-10 or more minute ones in 100% of cells as "3" (Figure 1). Levels "0" and "1" were considered to be negative, levels "2" and "3" positive. TRF2 protein expression was also assessed in the nuclei of the cells. Expression of the TRF2 protein was found positive when aggregates of TRF2 were present characterized as intensive red boddies. Nuclear staining was graded semiquantitatively. Complete absence of TRF2 protein aggregates was scored as "0"; their presence in 25% of cells or less was scored as "1", in more than 25% and less then 75% of cells as "2" and in 75% of cells and more as "3". In cell lines, the pattern of TRF2 expression was classified as large aggregates, small aggregates and diffuse granular pattern (Figure 2). Colocalization of TRF2 and PML was determined in 200 cells. The Gimp 2.0 program was been used to compose the pictures with PML and TRF2 protein expression. Colocalization of the proteins was present when aggregates of PML and TRF2 covered one over the other.

*Correlation of results with other characteristic.* Expression of PML protein was correlated with age of the patients, the status of microsatellite stability, localization of the tumor in the left or right colon or rectum, histology, TNM (Tumor, Nodi, Metastasis) stage and disease-free and overall survival.

*Microsatellite instability (MSI) analysis.* Genomic DNA was isolated from peripheral blood lymphocytes by a standard desalting method. DNA from paraffin-embedded tumor tissue was extracted using Nucleospin C+T kit (Macherey-Nagel, Düren, Germany). Paired normal and tumour DNA were used to amplify sequences (using PCR) of the following 4 mononucleotide and 5 dinucleotide microsatellite loci: BAT-RII, BAT-25, BAT-26, BAT-40, D2S123, D3S1029, D5S346, D17S250, D18S58 (Applera, Czech Republic). Fluorescent labeled fragments were analysed at ABI Prism 310 Genetic Analyzer. In most cases, multiplex analysis was used. Both tumor and nontumor fragments were compared and analysed for MSI and/or loss of heterozygosity (LOH). The presence of additional peaks in the PCR product from tumor DNA, not observed in DNA from normal tissue from the same patient was scored as instability at that particular locus. A tumor DNA that displayed instability in at least two of five loci or at least 30-40% of loci was scored as having high-frequency MSI (MSI-H), whereas a sample with no instability was scored as microsatellite stable (MSS). A tumor sample observed to have instability at one microsatellite locus was scored as having low-frequency MSI (MSI-L).

*Histological typing.* four categories were classified: adenocarcinoma grade 1 (low grade), 2 (medium grade) and 3 (high grade) and mucinous carcinoma.

The *TNM* (Tumor, Nodi, Metastasis) stage was classified according to the 6th edition of "TNM classification of malignant tumours" [29].

*Telomerase activity* was analysed by TRAPeze kit (Chemicon, Temecula, CA, USA) based on the TRAP (Telomeric Repeat Amplification Protocol) method in fresh frozen tumor samples [30]. In brief, the concentration of proteins in cell lysates was measured by the Bradford method and aliquots of the lysate containing 1ug of protein were loaded for the primer elongation and PCR. After separation on 12% polyacrylamide gel, telomerase ladder was visualised by Sybr Gold and CCD camera (Raytest, Straubenhardt, Germany).

*Statistical analysis.* Correlation of PML expression with age of the patients was tested using the ANOVA test, correlation with disease-free and overall survival using Kaplan-Meyer analysis and log rank test and with all the other characteristics using the chi-square test.

## Results

The patient and tumor characteristics as regards the age of patients, localisation and histological typing of the tumors and the TNM stages are provided in Tables 1-4.

In normal cells (stromal, normal colon crypts), the expression of the PML protein was at "2" or "3" levels. Expression of the PML protein in colorectal tumor samples with respect to MSI status is seen in Table 5. The difference in PML protein expression (negative, i.e. levels "0" and "1" versus pozitive, i.e. levels "2" and "3") between MSS and MSI-H tumors was not statistically significant (chi-square test, p=0.520). There was no correlation between PML expression and age of the patients (ANOVA, p=0.16), localization of the tumor in colon (chi-square test, p=0.07), histological typing of tumors (chi-square test, p=0.84), TNM classification (chi-square test, p=0.19, 0.93 and 0,61, respectively), disease-free

**Table No 1. PML protein expression with respect to age of the patients (ANOVA, p=0.16).**

| PML expression | No | Age | | | | |
|---|---|---|---|---|---|---|
| | | Minimum | Maximum | Median | Mean | Standard deviation |
| 1 | 18 | 23 | 77 | 53 | 52.22 | 14.78 |
| 2 | 42 | 24 | 77 | 43 | 45.67 | 15.23 |
| 3 | 20 | 27 | 80 | 49 | 52.50 | 16.14 |
| Total | 80 | 23 | 80 | 48 | 48.85 | 15.54 |

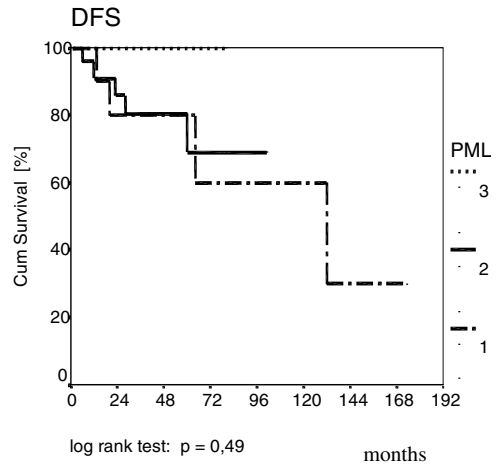


**Figure No 3. Disease-free survival of the patients with respect to PML protein expression; Kaplan-Meier analysis (log rank test: p=0,49).**

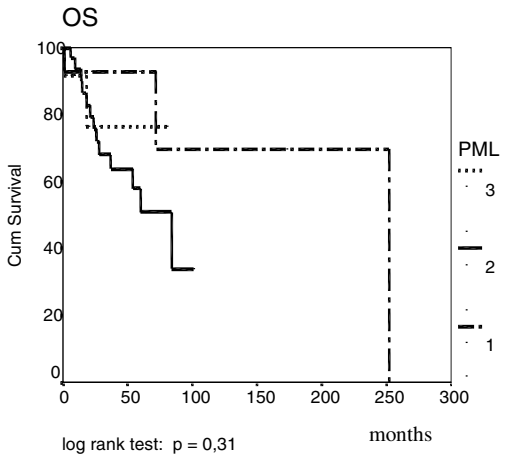


**Figure No 4. Overal survival of the patients with respect to PML protein expression; Kaplan-Meier analysis (log rank test: p=0,31).**

survival (log rank test, p=0.49; Figure 3) and overall survival (log rank test, p=0.31; Figure 4).

Results of PML and TRF2 expression in cell lines are summarized in Table 6. In Saos-2, U2OS and MCF7 cell lines, colocalization of TRF2 and PML was detected in 4, 7 and 15% of cells, respectively. In DU145, LNCaP, U87, HeLa and T98G cell lines, no colocalization of PML and TRF2 was found. Compared to Saos-2 and U2OS, accumulation of TRF2 was detected in a much lesser percentage of cells in DU145, HeLa and T98G cell lines. In LNCaP and U87 cell lines, TRF2 protein accumulation was not found (Figure 2).

Accumulation of the TRF2 protein was detected in 16 cases of 80 (20%) samples (6 MSI-H and 10 MSS cases). Colocalization of TRF2 with PML was detected in two MSI-H samples (i.e. 6% of these tumors). One of them was a tumor from a *hMLH1* germline mutation carrier, the other was sporadic. In two MSI-H tumors without TRF2 protein accumulation, from which fresh frozen tissue was available, detectable telomerase activity was found.

## Discussion

The PML protein is expressed in normal tissues, with the highest levels of the protein being found in postmitotic, differentiated cell types, such as endothelial cells, epithelial, and tissue macrophages, especially activated ones [31]. Downregulation of PML protein expression was detected in 22% of colorectal tumors compared to normal cells that express the protein at the "2" or "3" levels. Downregulation of the PML protein might represent a selective advantage for tumor cells. It is in accord with the needs of tumor cells to escape physiological processess of apoptosis, growth arrest and cellular senescence. The percentage of tumors with downregulation of PML protein expression is lower in our study compared to the study of Gurrieri and co-workers who found partial or complete loss of PML protein expression in 34 (31%) and 18 (17%) of 109 colon adenocarcinomas, respectively (32). They used immunohistochemistry to detect the protein. PML protein expression assessment was different from ours, being based on the number of PML bodies.

**Table No 2. PML protein expression with respect to the localization of the tumors in the colon (the data were not available for all patients; chi-square test, p=0.07).**

| PML expression | | Localization of tumors | | | |
|---|---|---|---|---|---|
| | | Left-sided | Rectum | Right-sided | Total |
| 1 | No | 7 | 2 | 8 | 17 |
| | % | 41.2% | 11.8% | 47.1% | 100% |
| 2 | No | 20 | 10 | 9 | 39 |
| | % | 51.3% | 25.6% | 23.1% | 100% |
| 3 | No | 2 | 5 | 7 | 14 |
| | % | 14.3% | 35.7% | 50.0% | 100% |
| Total | No | 29 | 17 | 24 | 70 |
| | % | 41.4% | 24.3% | 34.3% | 100% |

**Table No 3. PML protein expression with respect to the histological findings (chi-square test, p=0.84).**

| PML expression | | Histology | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | mucinous | Total |
| 1 | No | 6 | 8 | 2 | 1 | 17 |
| | % | 35.3% | 47.1% | 11.8% | 5.9% | 100% |
| 2 | No | 21 | 18 | 3 | 1 | 43 |
| | % | 48.8% | 41.9% | 7.0% | 2.3% | 100% |
| 3 | No | 9 | 8 | 3 | | 20 |
| | % | 45.0% | 40.0% | 15.0% | | 100% |
| Total | No | 36 | 34 | 8 | 2 | 80 |
| | % | 45.0% | 42.5% | 10.0% | 2.5% | 100% |

**Table No 4 a-c. PML protein expression with respect to TNM classification (the data were not available for all patients; chi-square test, p=0.19, 0.93 and 0.61 for T, N, M, respectively).**

a)

| PML expression | | T (Tumor) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | Total |
| 1 | No | | 4 | 6 | 5 | 15 |
| | % | | 26.7% | 40.0% | 33.3% | 100% |
| 2 | No | | 8 | 24 | 4 | 36 |
| | % | | 22.2% | 66.7% | 11.1% | 100% |
| 3 | No | 1 | 2 | 8 | 2 | 13 |
| | % | 7.7% | 15.4% | 61.5% | 15.4% | 100% |
| Total | No | 1 | 14 | 38 | 11 | 64 |
| | % | 1.6% | 21.9% | 59.4% | 17.2% | 100% |

b)

| PML expression | | N (Nodi) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | Total |
| 1 | No | 6 | 6 | 3 | | 15 |
| | % | 40.0% | 40.0% | 20.0% | | 100% |
| 2 | No | 17 | 10 | 6 | 1 | 34 |
| | % | 50.0% | 29.4% | 17.6% | 2.9% | 100% |
| 3 | No | 7 | 3 | 2 | | 12 |
| | % | 58.3% | 25.0% | 16.7% | | 100% |
| Total | No | 30 | 19 | 11 | 1 | 61 |
| | % | 49.2% | 31.1% | 18.0 | 1.6 | 100% |

c)

| PML expression | | M (Metastasis) | | |
|---|---|---|---|---|
| | | 0 | 1 | Total |
| 1 | No | 10 | 2 | 12 |
| | % | 83.3% | 16.7% | 100% |
| 2 | No | 28 | 8 | 36 |
| | % | 77.8% | 22.2% | 100% |
| 3 | No | 10 | 1 | 11 |
| | % | 90.9% | 9.1% | 100% |
| Total | No | 48 | 11 | 59 |
| | % | 81.4% | 18.6% | 100% |

Complete loss was defined as undetectable levels of PML and partial loss being defined by two or fewer PML nuclear bodies per cell. Especially the percentage of tumors with complete loss was relatively high in this study. The tumors classified as level "1" expression were characterized by one or two weak signals in less than 50% of the cells in our study. The likely explanation for this discrepancy might be in the used method with respect to the higher sensitivity of immunofluorescent staining used in our study. These signals might not be detected using immunohistochemistry. The other explanation might be false positivity caused by unspecific binding of the antibody. In any case, level "1" expression in our study can be characterized as severe downregulation of PML protein expression and we classed it as negative.

Loss of the PML protein is a frequent event in human cancers of various histologic origins. It is frequently lost in breast, lung, prostate, CNS, germ cell and thyroid tumors, non-Hodgkin's lymphomas and nasopharyngeal carcinoma [32-38].

**Table 5. PML protein expression with respect to the MSI status (chi-square test, p=0.520).**

| Intensity of PML expression | 0 | 1 | 2 | 3 | Total |
|---|---|---|---|---|---|
| MSI-H tumors | 0 | 7 (22%) | 15 (47%) | 10 (31%) | 32 (100%) |
| MSS tumors | 0 | 11 (22%) | 27 (57%) | 10 (21%) | 48 (100%) |

We tested the hypothesis that there might be a difference in PML protein expression between MSS and MSI-H tumors. These two pathogenetically distinct types of tumors are characterized by a series of differences [26-28]. However, no difference was found.

As for detection of ALT-associated PML bodies, Saos-2 and U2OS cell line served as a control, because they do not have active telomerase and are known to use the alternative mechanism of telomere elongation [16, 39]. The low 5% of cells showing APBs suggests that it is the G2 phase of the cell cycle where it is found [16]. DU145, LNCaP and HeLa cell lines have active telomerase [39, 40]. No colocalization of PML and TRF2 was found in these cell lines and generally accumulation of TRF2 was absent or much weaker then in Saos-2 or U2OS. Similar result were found in HeLa cells in other studies [16]. Surprising is the high percentage of APBs in MCF7 cell line, a telomerase-active one. MCF7 cell line was not studied for ALT and as coexistence of telomerase and ALT is biologically possible, we cannot exclude this fact. Another interesting observation is the presence of TRF2 accumulation. All the telomerase-positive cell lines had either no accumulation of TRF2 or accumulation present in only one quarter to one third of the cells compared to ALT-cell lines. Similar observation was made by Yeager et al 1999 with TRF1 nuclear aggregates being present in ALT positive cell lines and not detectable in telomerase-positive cell lines (16).

In paraffin-embedded tumor tissues, the percentage of cells available for analysis of colocalization was relatively low, about 20% of samples, due to the low number of samples with TRF2 protein expression. This might correspond to the above mentioned observation that TRF2 aggregates are not detectable or weakly expressed in a small proportion of cells in telomerase positive cell lines. Among TRF2 positive samples, colocalization of PML and TRF2 was found in two MSI-H tumors. Mismatch repair system has a central role in maintaining genomic stability by repairing DNA replication errors and inhibiting homologous recombination, i.e. recombination between non-identical sequences [41]. Telomeric and subtelomeric regions of chromosomes are not perfectly homologous both in yeast as well as in human cells [42]. As long term proliferation can be maintained by recombination between chromosome ends in the absence of

**Table 6. PML and TRF2 expression in cell lines (Abbreviations: ALT, alternative lengthning of telomeres; Tel, telomerase).**

| Cell line | Tissue of origin | PML (No. of bodies, size/% of cells) | TRF2 (No. of bodies, size/% of cells | Colocalisation of PML/TRF2 (% of cells) | Telomerase/ /ALT (citation) |
|---|---|---|---|---|---|
| **Saos-2** | Osteosarcoma | 2-5, minute/100% | 1-8, large/75% | 4% | ALT [16, 39] |
| **U2OS** | Osteosarcoma | 1-10, minute/100% | 1-10, large/80% | 7% | ALT [16, 39] |
| **DU145** | Prostate cancer | 3-10, medium/100% | 1-8, large/30%, otherwise diffuse granular expression | 0 | Tel [39, 40] |
| **LNCaP** | Prostate cancer | 4-10, minute/100% | Difuse granular expression | 0 | Tel [39, 40] |
| **U87** | Brain glioblastoma-astrocytoma | 4-10, minute/100% | Difuse granular expression | 0 | Tel [50] |
| **MCF7** | Breast adenokarcinoma | 1-8, large/80% | 1-8, large/80% | 15% | Tel [39] |
| **HeLa** | Cervical cancer | 2-10, minute/100% | 1-4, large/25%, otherwise diffuse granular expression | 0 | Tel [39, 40] |
| **T89G** | Glioblastoma | 10-20, minute/100% | 1-4, small/7%, otherwise diffuse granular expression | 0 | Tel [50] |

telomerase, the anti-recombination function of the mismatch repair system probably has an inhibitory effect on the ability of telomerase-defective cells to proliferate [17, 43]. Loss of mismatch-repair function has been shown to promote cellular proliferation in the absence of telomerase in yeasts. Enhanced telomeric recombination in human cells with mismatch repair defects might contribute to cell immortalization and hence tumorigenesis [17]. Tumors with microsatellite instability have been shown to be telomerase-positive in the same proportion as mismatch repair proficient tumors [44, 45]. Moreover, normal mucosa samples from HNPCC patients were positive for telomerase more often than samples from non-HNPCC patients suggesting that genetic defects in individuals with HNPCC syndrome facilitate the reactivation of telomerase activity, too [44]. Again, coexistence of ALT and telomerase seems to be biologically possible [46, 47].

An exact percentage of tumors using ALT is not known. About 10% of tumors are telomerase negative. However, not all these tumors display the telomere morphology characteristic of ALT, i.e. heterogeneous telomere length [48]. The relatively high proportion of tumors with colocalization of the PML and TRF2 proteins suggestive of ALT in this respect might be associated with a small number of samples analyzed. Using ALT-associated promyelocytic leukemia (PML) bodies detection by combined PML immunofluorescence and telomere fluorescence in situ hybridization, a relatively high proportion of ALT positive sarcomas and astrocytomas has been found (35% of 101 soft tissue sarcomas, 47% of 58 osteosarcomas , 34% of 50 astrocytomas) [49]. None of 17 papillary thyroid carcinomas was ALT positive [49].

In conclusion, we have found downregulation of PML protein expression in approximately 20% of colon tumors. There was no difference in PML protein expression between MSI-H and MSS tumors. There was no correlation between PML expression and any of the clinical characteristics in colon cancer suggesting lack of prognostic impact. TRF2 protein expression was detected in about 20% of samples. Colocalization of PML and TRF2, suggestive of the presence of ALT, has been found in 6% of MSI-H tumors.

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