Analysis of Central Regulatory Pathways in p53-deficient Primary Cultures of Malignant Fibrous Histiocytoma Exposed to Ifosfamide

THILO SCHLOTT¹, HELGE TAUBERT², AFSHIN FAYYAZI¹, STEFAN SCHWEYER¹, FRANK BARTEL², MONIKA KORABIOWSKA¹ and ULRICH BRINCK³

¹Department of Pathology and ²Department of Gastroenteropathology, Center for Pathology, Georg-August-University, D-37075 Goettingen; ³Department of Molecular Pathology, Institute of Pathology, Martin-Luther University, D-06097 Halle-Wittenberg, Germany

Abstract. Soft tissue sarcomas frequently carry p53 mutations reducing chemotherapeutical response. Especially malignant fibrous histiocytoma (MFH) reveals a reduced ifosfamide (IF) chemosensitivity when compared to other sarcoma entities. This is the first study to analyze MFH cells for the effects of IF on the expression of the pathways P16-CDK4-Rb and P14ARF-MDM2-P73 regulating cell cycle. The aim was to identify candidate genes possibly involved in the anti-apoptotic response of p53-deficient MFH cells during chemotherapy. PCR, real-time RT-PCR and confocal laser scanning microscopy were applied on primary cultures of MFH cells containing defective p53 genes. The cultures were treated with different concentrations of IF. A non-treated MFH culture served as negative control. A threshold concentration of IF (100 μM) was determined sparing the majority of the cells (99%), whereas higher IF quantities caused complete apoptosis. Data collected over a period of 48 h showed that the MFH cells surviving 100 μM IF overexpressed the kinase gene CDK4 and oncogene MDM2 by a factor of 63. A similar strong increase was observed at the protein level for both proteins. In contrast, the other proteins analyzed were not detectable. Additionally, the MFH cells induced complex patterns of MDM2 mRNA splicing and an abnormal mRNA transcript carrying a novel MDM2 missense mutation. These effects were neither observed in the non-treated culture nor in cultures completely inducing spontaneous apoptosis. Therefore, we speculate that the induction of the gene CDK4, and especially of MDM2, is involved in anti-apoptotic mechanisms of p53-negative MFH cells tolerating IF in vitro. Further experiments are necessary to test whether the novel candidate genes favor development of chemoresistance and whether MDM2 mRNA splicing variants contribute to this process in vivo.

In former clinical studies on sarcomas, ifosfamide (IF) proved to be a promising drug with a strictly dose-dependent activity (1). The compound is highly active in adults with high-grade soft-tissue sarcomas when applied in combination with etoposide (2). In general, chemotherapy affects the cell cycle and apoptosis, both of which are controlled by networks of interacting proteins. The most prominent networks are the P16-CDK4-Rb and the P14ARF-MDM2-p53 pathways, which were analyzed previously for certain genetic alterations in esophageal squamous cell carcinoma, vulvar carcinoma, multiple myeloma, astrocytic gliomas and osteosarcoma/Ewing sarcoma (3-7).

The P16-CDK4-Rb pathway is initiated by protein P16, also called the inhibitor A of CDK4 (INK4A), which in many tumors reveals homozygous deletions that inhibit G1 arrest (8). P16 binds with 1:1 stoichiometry to cyclin-dependent kinase CDK4 in competition with cyclin D and blocks the action of CDK4 to arrest the cell cycle (10). Rb is an essential regulator for cell division because the inhibition of Rb phosphorylation results in cell cycle arrest (11).

The P14ARF-MDM2-p53 pathway contains the potential tumor suppressor P14ARF protein which, similar to protein P16, is encoded by the human INK4a/ARF locus and connects the cyclin D/CDK4-Rb and the P14ARF-MDM2-P53 pathway (12). Nucleolar P14ARF was demonstrated to bind to human oncoprotein MDM2, thus inhibiting MDM2-dependent cell cycle progression (13). In contrast, unbound MDM2 promotes cell division by forming a complex with tumor suppressor p53 (14,15), which belongs to a family of homologue proteins.

Correspondence to: Thilo Schlott, Department of Pathology, Center for Pathology, Georg-August-University, D-37075 Goettingen, Germany. e-mail: tschlott@med.uni-goettingen.de

Key Words: Sarcoma, ifosfamide, real-time RT-PCR, P16-CDK4-Rb pathway, P14ARF-MDM2-p53-P73 pathway.
including protein P73 (16,17). This potential tumor suppressor reveals some of the p53 functions, such as transactivation and induction of cell cycle arrest/apoptosis; however, P73 activates only some of the p53-responsive promoters (18). Since the P73 protein isoform P73beta is bound by MDM2 in a p53-analogous manner (19), P73 is part of an analogous PI4ARF-MDM2-P73 pathway.

To our knowledge, none of the systems presented above has yet been analyzed by real-time RT-PCR and confocal laser scanning microscopy in MFH cells. The present study aimed at evaluating the modulating effects of chemotherapeutic drugs on central cell regulators in primary cultures of MFH tumor cells. Therefore, the complete pathways of genes were analyzed with regard to the effects of IF on mRNA and protein expression.

Materials and Methods

Cell cultures and ifosfamide treatment. A primary culture of malignant fibrous histiocytoma was isolated from fresh tissue and maintained in RPMI 1640 medium (PAA, Coelbe, Germany) containing 10% fetal calf serum (Biochrom, Germany) and an antibiotic solution, containing 100 units penicillin per ml and 50 µg streptomycin per ml (Biochrom). Cultures were grown in a humidified incubator containing 5% CO2 and used for incubation with IF (ASTA Medica Ag, Germany) at different concentrations including protein P73 (16,17). This potential tumor suppressor reveals some of the p53 functions, such as transactivation and induction of cell cycle arrest/apoptosis; however, P73 activates only some of the p53-responsive promoters (18). Since the P73 protein isoform P73beta is bound by MDM2 in a p53-analogous manner (19), P73 is part of an analogous PI4ARF-MDM2-P73 pathway.

To our knowledge, none of the systems presented above has yet been analyzed by real-time RT-PCR and confocal laser scanning microscopy in MFH cells. The present study aimed at evaluating the modulating effects of chemotherapeutic drugs on central cell regulators in primary cultures of MFH tumor cells. Therefore, the complete pathways of genes were analyzed with regard to the effects of IF on mRNA and protein expression.

Materials and Methods

Cell cultures and ifosfamide treatment. A primary culture of malignant fibrous histiocytoma was isolated from fresh tissue and maintained in RPMI 1640 medium (PAA, Coelbe, Germany) containing 10% fetal calf serum (Biochrom, Germany) and an antibiotic solution, containing 100 units penicillin per ml and 50 µg streptomycin per ml (Biochrom). Cultures were grown in a humidified incubator containing 5% CO2 and used for incubation with IF (ASTA Medica Ag, Germany) at different concentrations including protein P73 (16,17). This potential tumor suppressor reveals some of the p53 functions, such as transactivation and induction of cell cycle arrest/apoptosis; however, P73 activates only some of the p53-responsive promoters (18). Since the P73 protein isoform P73beta is bound by MDM2 in a p53-analogous manner (19), P73 is part of an analogous PI4ARF-MDM2-P73 pathway.

To our knowledge, none of the systems presented above has yet been analyzed by real-time RT-PCR and confocal laser scanning microscopy in MFH cells. The present study aimed at evaluating the modulating effects of chemotherapeutic drugs on central cell regulators in primary cultures of MFH tumor cells. Therefore, the complete pathways of genes were analyzed with regard to the effects of IF on mRNA and protein expression.

Materials and Methods

Cell cultures and ifosfamide treatment. A primary culture of malignant fibrous histiocytoma was isolated from fresh tissue and maintained in RPMI 1640 medium (PAA, Coelbe, Germany) containing 10% fetal calf serum (Biochrom, Germany) and an antibiotic solution, containing 100 units penicillin per ml and 50 µg streptomycin per ml (Biochrom). Cultures were grown in a humidified incubator containing 5% CO2 and used for incubation with IF (ASTA Medica Ag, Germany) at different concentrations including protein P73 (16,17). This potential tumor suppressor reveals some of the p53 functions, such as transactivation and induction of cell cycle arrest/apoptosis; however, P73 activates only some of the p53-responsive promoters (18). Since the P73 protein isoform P73beta is bound by MDM2 in a p53-analogous manner (19), P73 is part of an analogous PI4ARF-MDM2-P73 pathway.

To our knowledge, none of the systems presented above has yet been analyzed by real-time RT-PCR and confocal laser scanning microscopy in MFH cells. The present study aimed at evaluating the modulating effects of chemotherapeutic drugs on central cell regulators in primary cultures of MFH tumor cells. Therefore, the complete pathways of genes were analyzed with regard to the effects of IF on mRNA and protein expression.

Materials and Methods

Cell cultures and ifosfamide treatment. A primary culture of malignant fibrous histiocytoma was isolated from fresh tissue and maintained in RPMI 1640 medium (PAA, Coelbe, Germany) containing 10% fetal calf serum (Biochrom, Germany) and an antibiotic solution, containing 100 units penicillin per ml and 50 µg streptomycin per ml (Biochrom). Cultures were grown in a humidified incubator containing 5% CO2 and used for incubation with IF (ASTA Medica Ag, Germany) at different concentrations including protein P73 (16,17). This potential tumor suppressor reveals some of the p53 functions, such as transactivation and induction of cell cycle arrest/apoptosis; however, P73 activates only some of the p53-responsive promoters (18). Since the P73 protein isoform P73beta is bound by MDM2 in a p53-analogous manner (19), P73 is part of an analogous PI4ARF-MDM2-P73 pathway.

To our knowledge, none of the systems presented above has yet been analyzed by real-time RT-PCR and confocal laser scanning microscopy in MFH cells. The present study aimed at evaluating the modulating effects of chemotherapeutic drugs on central cell regulators in primary cultures of MFH tumor cells. Therefore, the complete pathways of genes were analyzed with regard to the effects of IF on mRNA and protein expression.
The cytoskeletal analysis reveals chromosomal changes indicating complete breakdown of the genome. As a result, the cells isolated from fresh MFH tissue were identified as tumor cells.

Sequence analysis of p53 and MDM2 PCR fragments. DNA and RNA PCR fragments were purified using the QIA Quick PCR Purification Kit (Qiagen), labelled with the PRISM Ready Reaction Dye Deoxy-TM Terminator Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany) and analysed in an ABI 310 analyzer.

Caspase-3 and May-Giemsa-Grunwald staining. Cytocentrifuged and fixed MFH cells were rinsed in TBS containing 10 % FCS and 0.3 % H₂O₂ for 10 min. Subsequently, the cells were incubated with the antibody against active caspase-3 (anti-caspase-3 rabbit anti-human IgG, R&D Systems, Wiesbaden, Germany) diluted 1:50 in Tris buffer for 30 min. After washing in TBS, the cells were further incubated with a biotin-conjugated polyclonal goat anti-rabbit antibody (DAKO, Denmark) for 20 min. Afterwards, the cells were incubated with HRP-conjugated streptavidin (DAKO) for 20 min. The antibodies were visualized with 3,3′diaminobenzidine (DAB). Additionally, Giemsa staining was performed. Cytocentrifuged cells were dried for 24 h, fixed in 100 % acetone for 10 min, stained with May-Giemsa-Grunwald and embedded in “SuperMount Medium” (DAKO). Apoptotic cells were identified by formation of apoptotic bodies.

Confocal laser scanning microscopy. For immunofluorescence microscopy, MFH cell cultures transferred to glass slides were analyzed on a Leica TCS confocal laser scanning system with a DM IRB microscope. In detail, a scanning modus x-y axis was used to horizontally section the cells, to study the distribution of fluorescence signals. Two different labelling techniques were used.
Figure 2. Analysis of p53 protein expression and nucleotide sequence in MFH cells treated with ifosfamide. A. Fluorescence microscopy using FITC-labelled antibody indicated that the sarcoma cells did not express the p53 protein. This result was also observed in untreated MFH cells. B. Sequence analysis of the underlying p53 gene. C. Nucleotide and derived amino acid sequence. Data prove that the lack of the p53 protein was caused by a 34 bp deletion in exon 7 of the p53 gene which shifted the reading-frame and induced a premature stop codon. In consequence, the gene codes for a truncated, unstable protein with abnormal amino acids at the C-terminus (bold amino acid letters).
Figure 3. Real-time RT-PCR for establishing standard curves that were used for quantification of β-actin, P14ARF, MDM2, P73, Rb, P16 and CDK4 gene expression in MFH cells. Each of the diagrams contains the simultaneous amplification of gene-specific PCR fragments (standard) in serial dilution (0.01, 0.1, 1.0, 10 and 100 atmol) over a period of 50 cycles. Additionally, the correlation coefficient and the efficiency of amplification reaction is presented in percent product per PCR run.
For single fluorescence labelling of cells with antibodies, coverslips were placed into cold methanol (100%, -20°C) for 5 min and into cold acetone (100%, -20°C) for 10 sec. Afterwards, cells were immediately placed into a glass container with PBS (pH 7.2) so that the slides were not allowed to dry. The cells were washed three times in PBS for several minutes. Excess PBS was removed with dry filter paper. The coverslips were placed on parafilm and then put into the wet chamber. The antibodies used were: Anti-P16 (50.1) sc-9968 mouse monoclonal IgG1, Anti-CDK4 (H-303) sc-749 rabbit polyclonal IgG, Anti-Rb (IF8) sc-102 mouse monoclonal IgG1, Anti-P14ARF (FL-132) rabbit polyclonal IgG, Anti-MDM2 1B10 (NCL-MDM2) mouse monoclonal IgM, Anti-P73 (H-79) sc-7957 rabbit polyclonal IgG and Anti-p53 (Pab 1801) sc-98 mouse monoclonal IgG1. The antibodies were obtained from Santa Cruz Biotechnology, Inc., California, USA, except MDM2 antibody (Novocastra, UK). Each antibody solution was diluted 1:50 in PBS buffer and applied onto the coverslips. Cells were incubated for 12 h and washed three times in PBS for 5 min on a laboratory shaker. Afterwards, the following secondary antibodies diluted in PBS 1:100 were applied: swine antibody anti-rabbit labelled with FITC (F205, DAKO), goat antibody anti-rabbit labelled with TRITC (Dianova, Hamburg, Germany), goat antibody anti-mouse labelled with TRITC (Dianova), and rabbit antibody anti-mouse labelled with FITC (F0261, DAKO). Cells were incubated with the antibodies for 60 min and washed three times in PBS. Finally, the cells were embedded in DAKO R fluorescent mounting medium (DAKO).

Analytical strategy of data evaluation. The complete set of experiments was performed in two separate runs in order to guarantee reproducibility of data. Both runs led to comparable results concerning mRNA expression profiles, level of protein expression, protein localisation and MDM2 mRNA splicing. Minor quantitative differences in the results (mostly below 5%) allowed us to use means of the quantitative results of both runs.

Results

Characterization of MFH primary cell culture. To confirm the genetic features of the primary culture obtained from fresh MFH tissue and to exclude the possibility that non-malignant, contaminating cells had been accidentally grown in the flasks,
Figure 6. Lack of apoptosis in IF-treated MFH cells treated with ifosfamide concentrations ≤100 μM. At t=48h immunocytochemical staining shows lack of caspase 3 expression in the cytoplasm of the cells (A). Simultaneously, May-Giemsa-Grunwald staining reveals lack of apoptotic bodies in the cultures (B).

Figure 7. Analysis of MDM2 mRNA splicing in primary culture of MFH cells surviving ifosfamide treatment. A. RT-PCR fragments separated on 2 % agarose gel. Lane A. Contains molecular weight marker. Lanes B to I correspond to t=0, 3, 6, 9, 12, 14, 24, 48h, respectively. The IF-treated MFH cells induce a heterogeneous pattern of mRNA transcripts. B. Sequence analysis of a transcript variant induced by the IF-treated cells (see the small, intense PCR product of about 300 bp in lanes D – I of part A1; the transcript is expressed from t=6h to t=48h). The exon boundaries are indicated by the bar. C. Nucleotide and amino acid sequence of the mRNA variant. The transcript carries a missense mutation GGC-GCC and lacks nucleotide sequence between exon 5 and exon 12. It codes for a truncated MDM2 protein carrying abnormal amino acids at the C-terminus (bold letters).
Figure 8. Localisation of MDM2 and CDK4 protein in MFH cells treated with 100 μM ifosfamide using confocal laser scanning microscopy, t=48h. A. MDM2 is strongly overexpressed and forms characteristic nuclear clusters in the cells. Note that this picture also contains a burst MFH cell releasing cytoskeletal structures which are associated with MDM2 proteins. B. At the same time the nucleus is densely filled with CDK4.
a cytogenetic test was performed. The cells grown from the fresh MFH tissue were indeed abnormal, showing a complex karyotype with many numerical and structural aberrations, many non-identified marker chromosomes and a ring chromosome (Figure 1). In detail, the results of karyotyping were: 63-68, XY, -X, -1, add(2)(p2), add(2)(p1), +del(2)(p1), del(3)(p12p23), inv(3)(q13p14), add(4)(p12)x2, +add(4)(p16), -6, add(6)(q1), add(7)(p11)x2, +del(7)(q32), -8,del(8)(p11), -9, add(9)(p2), add(9)(q34), del(12)(p11), add(12)(p11), -13, -14, der(1)(1;14)(p11;q11)x2, -15, -15, -16, -16, -17, del(17)(p1), del(18)(p11), der(18)del(18)(p11)add(18)(q23), -19,add(19)(q13), +20, -21, -22, -22, -22, +mar1, +mar2, +mar3, +mar4, +mar5, +3-7mar, +r(cp12).

Checking the genetic status of p53. The genetic status of p53 in MFH cultures was investigated by automatic sequencing. RT-PCR was used to amplify the p53 sequence covering the highly conserved central region from exon 5 to exon 8 of the published sequence (23). Sequencing demonstrated that MFH cells contained a 34 bp deletion in p53 exon 7 (Figure 2B) which shifted the reading frame. This result of RNA PCR was reaffirmed by DNA PCR using exon 7 sense and antisense primers (data not shown). As a result, the gene coded for a truncated, defective p53 protein carrying 16 abnormal amino acid residues near the C-terminus (Figure 2C). The alteration led to the loss of p53 protein, as could be demonstrated by confocal laser scanning microscopy (Figure 2A).

Real-time RT-PCR. Following quantitative PCR analyses of the genes β-actin, P16, CDK4, Rb, P14ARF, MDM2 and P73, the reactions were checked by standard curve analysis. As a result, the templates were amplified with high efficiency (Figure 3). Furthermore, the PCR fragments were separated by agarose gel electrophoresis. The data indicated that specific PCR fragments of the expected length had been produced (Figure 4). In MFH cells treated with 100 μM IF, MDM2 mRNA was up-regulated by a factor of 63, P14ARF mRNA by a factor of 9.2, CDK4 by a factor of 63 and Rb mRNA by a factor of 8 (Figure 5). Simultaneously, P73 mRNA and P16 mRNA were neither up-regulated nor expressed according to the very low mRNA values (<0.00006 attomol). The highest increase of gene expression per period of time was registered for CDK4, where mRNA increased by a factor of 38 from t=24h to t=48h (Figure 5). However, the changes described above were only observed in MFH cells during treatment with 100 mM IF. MFH cultures treated with a higher IF concentration as well as non-treated MFH cells did not show any expressional variations.

Caspase-3 and May-Giemsa-Grunwald staining. The anti-apoptotic reactions of the MFH cell cultures were checked using caspase-3 and May-Giemsa-Grunwald staining. Immunochemical signals indicate that the MFH cells surviving incubation with 100 mM IF revealed a weak caspase-3 staining and the percentage of apoptotic cells less than 1 % (Figure 6).

Analysis of MDM2 mRNA splicing. The changes in gene expression observed in MFH cells treated with 100 μM IF were associated with a varying, complex spectrum of MDM2 mRNA splicing transcripts induced at t=6h (Figure 7A, lane D). In contrast, the untreated MFH cells and the MFH cells treated with another IF concentration only expressed the full-length form of MDM2 mRNA. Notably, sequence analysis proved that the IF-surviving MFH cells contained an abnormal MDM2 splicing variant (Figure 7B). It was expressed from t=6h to t=48h in separate experimental runs and coded for a truncated MDM2 protein consisting of 57 correct amino acids and the incorrect sequence V-D-Q-F-K before the premature stop codon (Figure 7C). Additionally, this mRNA transcript contained a MDM2 mutation which has not been described so far and which was located at nucleotide position (amino acid residue) 484 (58) in exon 5 of the published sequence (14) near the exon 5/12 boundary. The alteration changed codon GGC to GCC, thus replacing amino acid Gly by Ala (Figure 7C) and was not detected in the full-length MDM2 mRNA.

Protein expression. According to the results of confocal laser scanning microscopy, synthesis of CDK4 and MDM2 protein was initiated in cells treated with 100 μM IF parallel to the up-regulation of the underlying mRNA. The MFH cells revealed intense nuclear accumulation of MDM2 (Figure 8A) and CDK4 (Figure 8B), respectively. It should be noted that the MDM2 signals also indicated association with filamentous structures clustered around the nuclei of IF-treated MFH cells. Although their corresponding mRNA values increased (by a factor of 8 and 9.2, respectively) and reached the highest values at t=48h, the tumor suppressor proteins Rb and P14ARF were not expressed in the surviving cells (as a matter of fact the Rb and P14ARF fluorescence was difficult to differentiate from the background fluorescence; data not shown). The P16, p53 and P73 proteins were not detected in treated and non-treated MFH cells throughout the experiment.

Discussion

Successful chemotherapy of human soft tissue sarcomas is a result of both the lack of drug resistance as well as the inhibition of genes promoting cell cycle progression. This is the first study to analyze a network of the cell cycle regulators P16, CDK4, Rb, P14ARF, MDM2 and P73 using real-time RT-PCR during chemotherapy of MFH cells. Since many sarcomas contain gene mutations which inactivate
tumor suppressor p53 (24,25) and which can reduce the efficiency of chemo- or radiotherapy of sarcomas (26,27), an MFH culture was selected which contained defective p53 gene. This type of soft tissue tumor is usually treated with a protocol based on the therapeutic drug ifosfamide (IF). IF treatment is a non-natural way of modulating cell physiology and could be associated with some novel features that are not normally observed. In fact, the initial physiological effects of IF incubation observed in surviving MFH cultures were a dense clustering of MDM2 protein in the nucleoplasm of MFH cells and an association of MDM2 with structures similar to cytoskeletal fibers. The latter finding is puzzling and merits further attention. In fact, the MDM2-associated protein p53 has already been shown to bind to the microtubules following DNA damage, which serves as a means of transport to the nucleus (28). Therefore, it is possible that IF-induced DNA damage induced attachment of p53-MDM2 complexes to the cytoskeleton.

On comparing the activities of six different regulatory genes, distinct changes occurred in the MFH cells tolerating critical IF concentration. They were found at the mRNA/protein level and may indicate a potential anti-apoptotic response of cell cycle genes. According to the data, the MFH cells initiated the expression of the gene CDK4 and oncogene MDM2, thus probably promoting cell cycle progression. However, it is a striking finding that surviving MFH cells simultaneously up-regulated the oncogene MDM2 gene to such an extent that MDM2 mRNA dominated the pool of the mRNAs analyzed. Simultaneously, important MDM2 inhibitors such as P14ARF protein and P73 protein were not expressed for compensating MDM2 function. In summary, our data support the hypothesis that the expression of the P16-INK4A-Rb and P14ARF-MDM2-P73 systems is imbalanced as a response to the chemotherapeutic drug IF, thus allowing MDM2 and CDK4 to develop their pro-proliferative and anti-apoptotic functions.

Notably, the present study analyzed surviving MFH cells that induced MDM2 splicing variants and gene mutation. The novel mutation was located at amino acid position 58 and detected in an abnormal splicing transcript among non-mutant full-length transcripts. Since the mutation was only detected in the first run of the parallel experiments (the non-mutant form was also found in the second run), one may speculate that a subpopulation of MFH cells may have developed the mutation under the influence of ifosfamide. Different kinds of MDM2 missense mutations have already been described for the osteosarcoma cell line at amino acid positions 111, 139, 159, 218, 231, 308, 319 and 435, respectively (30). In the present study, the mutation caused a Gly→Ala conversion in the p53-binding domain between amino acid 42 and 94 of the MDM2 protein, possibly influencing the secondary structure and regulatory function. It is remarkable that the mutant transcript was exclusively expressed in the MFH cell culture incubated with 100 mM IF and that it was induced from t=6h until the end of the experiment. Further in vitro experiments are necessary to determine the role of this mutant mRNA transcript in MDM2 overexpression and cell survival. Since a confusing pattern of MDM2 mRNA splicing was previously reported for soft tissue sarcomas (30,31), and was also induced in the primary MFH culture, it is also possible that the MFH cells are able to perform an IF-dependent anti-apoptotic “switch” in mRNA splicing.

In summary, CDK4 and MDM2 were identified as novel candidate genes possibly working against IF-dependent apoptosis in vitro. We propose that this process could also include the formation of abnormal MDM2 proteins coded by normal and mutant mRNA transcripts resulting from an anti-apoptotic switch. Further functional studies must clarify whether the candidate genes and variants of MDM2 mRNA play a role in the IF-dependent chemoresistence of MFH tumors.

Acknowledgements

The authors are grateful to Ms. Melanie Gebhardt, Department of Cytopathology, Georg August University Goettingen, Germany, for her excellent technical assistance. This study was supported by Grant 10-1728 from the Dr. Mildred Scheel Foundation, Germany.

References