Overexpression of Inosine 5’-Monophosphate Dehydrogenase Type II Mediates Chemoresistance to Human Osteosarcoma Cells

Jörg Fellenberg*, Pierre Kunz*, Heiner Sähr, Daniela Depeweg

Department of Experimental Orthopedics, Orthopedic University Hospital Heidelberg, Heidelberg, Germany

Abstract

Background: Chemoresistance is the principal reason for poor survival and disease recurrence in osteosarcoma patients. Inosine 5’-monophosphate dehydrogenase type II (IMPDH2) encodes the rate-limiting enzyme in the de novo guanine nucleotide biosynthesis and has been linked to cell growth, differentiation, and malignant transformation. In a previous study we identified IMPDH2 as an independent prognostic factor and observed frequent IMPDH2 overexpression in osteosarcoma patients with poor response to chemotherapy. The aim of this study was to provide evidence for direct involvement of IMPDH2 in the development of chemoresistance.

Methodology/Principal Findings: Stable cell lines overexpressing IMPDH2 and IMPDH2 knock-down cells were generated using the osteosarcoma cell line Saos-2 as parental cell line. Chemosensitivity, proliferation, and the expression of apoptosis-related proteins were analyzed by flow cytometry, WST-1-assay, and western blot analysis. Overexpression of IMPDH2 in Saos-2 cells induced strong chemoresistance against cisplatin and methotrexate. The observed chemoresistance was mediated at least in part by increased expression of the anti-apoptotic proteins Bcl-2, Mcl-1, and XIAP, reduced activation of caspase-9, and, consequently, reduced cleavage of the caspase substrate PARP. Pharmacological inhibition of IMPDH induced a moderate reduction of cell viability and a strong decrease of cell proliferation, but no increase in chemosensitivity. However, chemoresistant IMPDH2-overexpressing cells could be resensitized by RNA interference-mediated downregulation of IMPDH2.

Conclusions: IMPDH2 is directly involved in the development of chemoresistance in osteosarcoma cells, suggesting that targeting of IMPDH2 by RNAi or more effective pharmacological inhibitors in combination with chemotherapy might be a promising means of overcoming chemoresistance in osteosarcomas with high IMPDH2 expression.

Introduction

Osteosarcoma is the most common primary malignant tumor of bone, typically affecting the long tubular bones of children and adolescents. The prognosis of high-grade osteosarcoma treated with surgery alone has been very poor, with 5-year survival rates below 20% [1]. Major advances in treatment over the past three decades, in particular the introduction of neoadjuvant chemotherapy, have markedly improved the outcome, with long-term relapse-free survival rates ranging from 55% to 75% [2,3]. However, the remainder of patients respond poorly to chemotherapy with an increased risk of relapse and the development of metastasis. Further efforts to improve patient outcome, for example by means of novel treatment protocols, have not significantly affected overall and disease-free survival of osteosarcoma patients over the past 20 years [4,5]. The lack of responsiveness to chemotherapy due to intrinsic or acquired chemoresistance is the major reason for poor survival and disease relapse of osteosarcoma patients. However, the mechanisms underlying osteosarcoma chemoresistance remain largely unknown. Therefore, the identification of prognostic factors that allow risk stratification at the time of diagnosis and elucidation of the mechanisms underlying chemoresistance will be pivotal in the development of new therapeutic strategies. In a previous study we identified IMPDH2 (inosine monophosphate dehydrogenase, type II) as independent prognostic factor for the response to chemotherapy in osteosarcoma patients. IMPDH2 gene expression was significantly elevated in patients with poor response and significantly associated with poor event-free survival [6].

IMPDH encodes the rate-limiting enzyme in de novo guanine nucleotide biosynthesis, maintaining the cellular guanine deoxyribonucleotide and ribonucleotide pools needed for DNA and RNA synthesis. IMPDH has been linked to cell growth, differentiation, and malignant transformation [7–10]. Two isoforms of IMPDH
have been described. Type I is constitutively expressed in normal cells, whereas type II activity has been shown to be increased in proliferating and especially malignant cells [10–11]. Thus, IMPDH has been considered an attractive target for immunosuppression as well as antiviral and cancer therapy [12–15]. IMPDH inhibitors such as tiazofurin and benzamide riboside have been shown to induce terminal differentiation in a variety of human cancer cells [16,17] and have been successfully applied in clinical trials [18,19]. Furthermore, IMPDH2 has been shown to be overexpressed in methotrexate (MTX)-resistant erythroleukemia K562 and human colon cancer cells. Pharmacological inhibition of IMPDH sensitized these cells to MTX treatment, suggesting that IMPDH might be a target for the modulation of chemosensitivity [20,21].

The aim of the present study was to investigate whether IMPDH2 is directly involved in the development of chemoresistance in osteosarcomas and whether inhibition of IMPDH2 activity or gene expression might usefully improve the outcome of therapy.

Our results demonstrate that IMPDH2 overexpression induces a strong chemoresistance in osteosarcoma cells which is mediated at least in part by increased expression of anti-apoptotic proteins. Although IMPDH2 knock-down or pharmacological inhibition of IMPDH2 enzyme activity did not significantly influence the chemosensitivity of wild-type osteosarcoma cells, chemoresistant IMPDH2-overexpressing Saos-2 cells were resensitized by IMPDH2 knock-down.

Results

The observation in our previous study of frequent IMPDH2 overexpression in osteosarcoma patients with poor response to chemotherapy and the identification of IMPDH2 as an independent prognostic marker for chemotherapy response suggest that IMPDH2 might be directly involved in the development of chemoresistance. To verify this hypothesis we established osteosarcoma cell lines with modulated IMPDH2 expression either by overexpression of the IMPDH2 coding sequence in Saos-2 cells (Saos-2 cdsIMPDH2) or by IMPDH2 knock-down using an shRNA construct specific for IMPDH2 (Saos-2 shIMPDH2). Western blot analysis of IMPDH2 protein expression in these cell lines showed a marked increase of IMPDH2 expression in Saos-2 cdsIMPDH2 cells and a considerable knock-down of IMPDH2 protein expression in Saos-2 shIMPDH2 cells compared to wild-type cells and cells stably transfected with the empty vector (Fig. 1A).

The analysis of chemosensitivity revealed a strong resistance of IMPDH2-overexpressing Saos-2 cdsIMPDH2 cells against cisplatin and methotrexate (Fig. 1B+C). Concerning the calculated IC50 values, overexpression of IMPDH2 induced 118-fold resistance against cisplatin and 14-fold resistance against methotrexate compared to Saos-2 wild-type cells (Table 1). Contrary to our expectations, IMPDH2 knock-down did not enhance the chemosensitivity of Saos-2 cells (Fig. 1B+C). At high MTX concentrations Saos-2 shIMPDH2 cells even showed a slightly more resistant phenotype rather than the expected sensitive phenotype. We assume that the reduced proliferation rate of IMPDH2 knock-down cells influences the susceptibility of these cells to cytotoxic drugs and that this effect is more pronounced for MTX, which acts much more slowly than cisplatin.

As chemotherapeutic drugs are known to exert their effects mainly through the activation of the mitochondrial apoptosis pathway, we further analyzed the expression of several key players in this pathway in cisplatin-treated Saos-2 wild-type and Saos-2 cdsIMPDH2 cells by western blotting. Cleavage of poly-ADP-ribose polymerase (PARP), a downstream substrate of caspase-9, was markedly reduced in IMPDH2-overexpressing cells. Furthermore, the cleavage and therefore the activation of caspase-9 was strongly reduced in these cells (Fig. 2). In addition, untreated Saos-2 cdsIMPDH2 cells showed increased expression of the anti-apoptotic mitochondrial proteins Bcl-2 and Mcl-1 compared to Saos-2 wild-type cells. Upon cisplatin treatment Bcl-2 expression was upregulated in Saos-2 wild-type cells while it was downregulated in IMPDH2-overexpressing cells. Mcl-1 expression was
The effects of inhibition of IMPDH2 enzyme activity on cell viability and chemosensitivity were evaluated using mycophenolic acid (MPA), an IMPDH inhibitor with a five times higher affinity to IMPDH2 than to the isoform IMPDH1. MPA alone induced concentration-dependent cell death in Saos-2 wild-type, Saos-2 pCMS, and Saos-2 shIMPDH2 cells. In Saos-2 cdsIMPDH2 cells only the highest MPA concentration induced a slight decrease in cell viability. However, MPA did not influence the chemosensitivity of any of the tested cell lines to cisplatin (Fig. 3). As the susceptibility of cells to treatment with chemotherapeutic drugs is highly dependent on cell growth, we analyzed the effects of MPA on cellular proliferation. MPA inhibited cell proliferation in a concentration-dependent manner in all tested Saos-2 cell lines (Fig. 4A).

To verify that the observed antiproliferative effect of MPA was due to a depletion of guanine nucleotides caused by the inhibition of IMPDH, Saos-2 wild-type cells were treated with MPA with or without the addition of guanine. Guanine almost completely abolished the antiproliferative effects of MPA, indicating a specific inhibition of IMPDH enzyme activity (Fig. 4B).

Since the pharmacological inhibition of IMPDH2 did not influence the chemoresistance of IMPDH2-overexpressing Saos-2 cells, we analyzed whether reduction of IMPDH2 gene expression is sufficient to provoke resensitization of these cells. For this purpose we cotransfected Saos-2 cdsIMPDH2 cells with three different shRNA constructs specific for IMPDH2 and generated stable cell lines. IMPDH2 mRNA levels were 52–60% lower in these cell lines than in the parent Saos-2 cdsIMPDH2 cells (Fig. 5A). Upon treatment with cisplatin, cotransfection of shIMPDH2 significantly decreased the amount of viable cells compared to Saos-2 cdsIMPDH2 cells. At high cisplatin concentrations the chemosensitivity of these cells was comparable to that of Saos-2 pCMS control cells (Fig. 5B).

**Discussion**

The existence or development of intrinsic or acquired chemoresistance represents the principal reason for poor survival and disease recurrence in osteosarcoma patients. Unfortunately, the mechanisms underlying osteosarcoma chemoresistance remain largely unknown. However, knowledge of the mediators that contribute to chemoresistance is pivotal to the identification of high-risk patients and the development of new therapeutic strategies. In order to identify prognostic factors for chemotherapy response we previously screened osteosarcoma cell lines for drug-regulated genes. Among other genes, we observed upregulation of IMPDH2 in response to cytotoxic drugs [22]. We further screened osteosarcoma biopsies for IMPDH2 gene expression and correlated these data with the patients’ response to chemotherapy as well as their overall and event-free survival. Expression of IMPDH2 was frequently increased in the subgroup of patients with poor response to chemotherapy and turned out to be an independent prognostic factor significantly associated with chemotherapy response and event-free survival [6]. These data suggested that IMPDH2 might be directly involved in the development of chemoresistance. In fact, the findings of the present study demonstrate that overexpression of IMPDH2 in osteosarcoma cells induces strong chemoresistance to cisplatin and methotrexate, two drugs frequently used for osteosarcoma therapy. As chemotherapeutic drugs exert their cytotoxic effects mainly through the activation of the mitochondrial apoptosis pathway, we investigated the expression of several apoptosis-related factors. Untreated IMPDH2-overexpressing Saos-2 cells displayed increased expres-

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**Table 1.** IC50 values of cisplatin and methotrexate in different Saos-2 cell lines.

|        | Cisplatin (72 h, µg/ml) | Methotrexate (96 h, ng/ml) |
|--------|------------------------|---------------------------|
| Saos-2 wild-type | 2.49±0.01 | 17.72±1.7 |
| Saos-2 pCMS | 2.11±0.70 | 16.97±1.1 |
| Saos-2 cdsIMPDH2 | 295±26 | 245±55 |
| Saos-2 shIMPDH2 | 2.61±0.75 | 32.13±12 |

Values are presented as mean ± SEM.

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**Figure 2.** Western blot analysis of different apoptosis-related proteins in Saos-2 wild-type cells and Saos-2 cdsIMPDH2 cells after treatment with cisplatin (2.5 µg/ml) at the indicated time points.

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sion of the anti-apoptotic proteins Bcl-2 and Mcl-1 as well as upregulation of XIAP upon cisplatin treatment. As a consequence, activation of caspase-9 and cleavage of the caspase substrate PARP were markedly reduced in these cells upon treatment with cisplatin, demonstrating IMPDH2-mediated inhibition of the mitochondrial apoptosis pathway. Interestingly, the pro-apoptotic mitochondrial proteins Bak and Bax have been shown to play an important role in the regulation of apoptotic cell death which is strongly associated with the depletion of the intracellular GTP pool [23]. Thus, the IMPDH-dependent regulation of intracellular GTP-pools seems to be crucial for the balance of anti- and pro-apoptotic proteins, which for their part influence the susceptibility of the cell to apoptotic stimuli, including cytotoxic drugs.

As catalyst of the rate-limiting step in the de novo synthesis of guanine nucleotides, IMPDH2 has been identified as an important regulator of cell proliferation [9]. In particular, proliferating lymphocytes are strongly dependent on the de novo synthesis of nucleotides, making IMPDH an attractive target for immunosuppressive therapies. IMPDH inhibitors such as MMF and its active compound MPA are thus widely used as immunosuppressive agents. Likewise, rapidly proliferating neoplastic cells are characterized by a high demand on IMPDH-mediated nucleotide synthesis. Expression of IMPDH2, particularly the type II isoform, has been shown to be significantly increased in many types of malignancies, making IMPDH2 also an attractive target for cancer therapy [10–12,24,25]. Several studies have already demonstrated the cytotoxic effects of IMPDH inhibitors and their high potential as anticancer drugs [26–28]. Phase II/III trials of IMPDH inhibitors such as tiazofurin and benzamide riboside have been conducted with very promising results, although studies were terminated due to neurotoxic side effects [18,19,29]. Although the essential role of IMPDH in cancer cell proliferation has been extensively studied, little is known about the involvement of this enzyme in the regulation of chemosensitivity. Increased IMPDH mRNA levels have been detected in MTX-treated and MTX-resistant human colon cancer and erythroleukemia cells. Inhibition of IMPDH significantly increased the sensitivity of the resistant cell lines to MTX, indicating that targeting IMPDH might constitute a promising means of minimizing the development of resistance [20,21]. We did not detect an increase in chemosensitivity in osteosarcoma cells treated with the IMPDH inhibitor MPA. Likewise, knock-down of IMPDH2 gene expression did not sensitize Saos-2 wild-type cells to cisplatin or methotrexate. Because of these unexpected observations and the fact that cytotoxic drugs preferentially act on rapidly proliferating cells, we assumed that the antiproliferative actions of IMPDH2 inhibitors counteract their effects on chemosensitivity. In fact, MPA induced a strong dose-dependent decrease in cell proliferation with an expected higher sensitivity of IMPDH2 knock-down cells and a lower sensitivity of IMPDH2-overexpressing cells. Besides cell proliferation, the p53 status of the analyzed Saos-2 cells might contribute to the failure of MPA to induce chemosensitivity. Saos-2 cells have a p53 null genotype and were chosen because p53 mutations and p53 inactivation are common features of osteosarcomas. The p53 gene functions as a key regulator of the apoptotic program. It is activated in response to cellular stress and exerts its well-documented pro-apoptotic functions mainly in a transcription-dependent manner. The IMPDH inhibitor MPA has been shown to activate and stabilize p53, which in turn mediates cell cycle arrest and apoptosis in response to guanine nucleotide depletion [30–32]. These data suggest that a functional p53 pathway is required for the induction of apoptosis in response to nucleotide depletion caused by IMPDH inhibitors. While

Figure 3. Chemosensitivity of Saos-2 wild-type, Saos-2 pCMS, Saos-2 shIMPDH2, and Saos-2 cdsIMPDH2 cells treated with the IMPDH inhibitor mycophenolic acid (MPA) at the indicated concentrations for 72 h with or without cisplatin (2.5 μg/ml). Analyses were performed in triplicate and the results are presented as mean ± SD. doi:10.1371/journal.pone.0012179.g003
pharmacological inhibition of IMPDH2 activity did not significantly alter the chemosensitivity of the analyzed cells, IMPDH2-overexpressing cells could be resensitized by cotransfection of an IMPDH2-specific shRNA construct. In association with the observed decrease in IMPDH2 gene expression, sensitivity to cisplatin increased, reaching the sensitivity levels of control cells at high cisplatin concentrations.

Altogether, we were able to demonstrate the induction of strong chemoresistance in osteosarcoma cells by overexpression of IMPDH2. The observed chemoresistance is mediated at least in part by upregulation of anti-apoptotic proteins, leading to inhibition of the mitochondrial apoptotic signaling pathway. Pharmacological inhibition of IMPDH2 did not enhance chemosensitivity, probably due to the strong antiproliferative effects of the inhibitor or the absence of functional p53. However, reduction of IMPDH2 gene expression in IMPDH2-overexpressing cells resensitized these cells to cytotoxic drugs. As IMPDH2 overexpression is frequently observed in osteosarcoma patients with poor outcome, these data suggest that IMPDH2 might be a novel therapeutic target in this disease.

Figure 4. Influence of the IMPDH inhibitor mycophenolic acid (MPA) on cell proliferation of different Saos-2 cell lines. A: MPA was added to the culture medium at the indicated concentrations and cell proliferation was determined using WST-1 assay at 0, 24, 48, and 72 h. B: Saos-2 wild-type cells were treated with the indicated concentrations of MPA for 48 h with or without the addition of 10 μM guanine. Cell proliferation was analyzed by WST-1 assay at 450 nm. Analyses were performed in triplicate and the results are presented as mean ± SD.

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response to chemotherapy, targeting of IMPDH2 by RNAi or more effective inhibitors in combination with chemotherapy might provide an effective synergistic treatment to overcome chemoresistance in osteosarcomas. It is clear that this hypothesis is still at a very early stage of development but add an interesting point of discussion and explanation of the observed effects.

Materials and Methods

Cell culture and drug treatment
The human osteogenic sarcoma cell line Saos-2 was originally obtained from the American Type Culture Collection (Rockville, USA). Cells were maintained in RPMI 1640 (Lonza GmbH, Wuppertal, Germany) containing 2 mM L-glutamine and 25 mM HEPES and supplemented with fetal calf serum (Biochrom, Berlin, Germany), and 100 U/ml penicillin/streptomycin (Lonza GmbH) at 37°C in a humidified 5% CO₂ atmosphere. Cells were kept in a logarithmic growth phase and split following treatment with trypsin/EDTA (Lonza GmbH), washed in PBS, and replated in fresh culture medium. For drug treatment, cisplatin (Sigma-Aldrich, Taufkirchen, Germany) was dissolved in N,N-dimethylformamide at a concentration of 15 mg/ml and added to the culture medium in the indicated concentrations. Methotrexate was dissolved in 0.01N NaOH and further diluted in PBS to a concentration of 10 mg/ml. Mycophenolic acid (Sigma-Aldrich) was dissolved in methanol at a concentration of 50 mM and added to the culture medium in the indicated concentrations.

Transfection and generation of stable cell lines
For overexpression of IMPDH2 the full coding sequence of IMPDH2 was cloned into the mammalian expression vector pCMS-EGFP (Clontech, Germany) and verified by sequencing. For gene knock-down a commercially available shRNA expression
vector expressing the following IMPDH2-specific 29-mer shRNA was used: 5'-ATAAGCTTCATTGCTGTATGGAGAGCCGCTT-3' (Origene, Rockville, USA). In both cases Saos-2 cells were transfected by electroporation using 10^6 cells and 0.5 μg plasmid and the microprotator MP-100 (PeqLab, Erlangen, Germany). Forty-eight hours after transfection, medium was replaced by fresh medium containing 600 μg/ml gentamicin (Sigma-Aldrich) for cells transfected with the pCMS-EGFP vector or 1 μg/ml puromycin (Sigma-Aldrich) for cells transfected with shRNA constructs. Stable clones were selected for at least 4 weeks before single colonies were picked and analyzed for IMPDH2 expression by quantitative PCR and western blot.

Western blot analysis
Cells were washed in PBS, lysed in RIPA buffer (Santa Cruz, Heidelberg, Germany) containing protease inhibitor cocktail (Santa Cruz), incubated for 1 h at 4°C, and centrifuged at 12000 g for 10 min, to remove cellular debris. Total protein concentrations were determined by BCA-assay (Pierce, Rockford, USA), and 10 μg of total protein was subjected to gel electrophoresis. Proteins were separated on a 10% polyacrylamide gel and transferred to Immobilon-P membranes (Millipore, Schwalbach, Germany). After blocking in PBS supplemented with 5% skim milk (Sigma-Aldrich) and 0.05% Tween 20 (Sigma-Aldrich) membranes were incubated overnight at 4°C with one of the following primary antibodies at the indicated dilutions: IMPDH2 (1:500) (Atlas Antibodies, Stockholm, Sweden), PARP (1:500) (Promega, Mannheim, Germany), caspase-9 (1:1000) (Promega, Mannheim, Germany), mcl-1 (1:250) (Santa Cruz), XIAP (1:250) (Calbiochem, San Diego, USA), bcl-2 (1:250) (Cell Signaling Technology, Danvers, USA), p85 (1:1000) (Promega, Mannheim, Germany), caspase-9 (1:1000) (Cell Signaling Technology, Danvers, USA), and actin (1:5000) (BD Transduction Laboratories, Heidelberg, Germany). After incubation with the primary antibody, membranes were washed three times with PBS containing 0.1% Tween 20 and incubated for 1 h at room temperature with 5000-fold diluted peroxidase conjugated goat anti-rabbit IgG (Santa Cruz) or goat anti-mouse IgG. Proteins recognized by the antibody were visualized with LumiLight western blotting substrate (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions.

Quantitative PCR
Two micrograms of total RNA extracted with RNaseasy Mini kit (Qiagen, Hilden, Germany) were reverse transcribed using Sensi-Script (Qiagen) and 10 μM oligo-dT primer for 2 h at 37°C in a total volume of 20 μl. Quantitative real-time PCR was performed in a LightCycler instrument (Roche Diagnostics) in a total volume of 20 μl using the Absolute SYBR Capillary mix (Thermo Scientific, Dreieich, Germany) and 1 μl of cDNA as template. Samples were heated to 95°C for 15 min followed by 40 cycles of denaturation at 95°C for 3 s, annealing at 60°C for 15 s, and extension at 72°C for 20 s. After the last cycle, a melting curve analysis was performed to verify the specificity of the amplified PCR products. The amount of PCR product was calculated using an external standard curve and LightCycler software. Calculated gene expressions were normalized on the basis of the β-actin (ACTB) expression in the corresponding samples.

Analysis of chemosensitivity and cell proliferation
Cell viability was quantified by propidium iodide staining and subsequent flow-cytometric analysis. For drug treatment 7.5×10^4 cells were seeded in a 24-well plate 24 h before treatment. After incubation for the indicated time, adherent and detached cells were collected, washed in PBS, centrifuged, and resuspended in PBS supplemented with 1% FCS and 2.5 μg/ml propidium iodide (Invitrogen, Karlsruhe, Germany). Samples were directly analyzed on a FACSscalibur cytometer using the CellQuest software (Becton Dickinson, Hamburg, Germany).

For the analysis of cell proliferation 2.5×10^5 cells were seeded in a 96-well plate. After 0, 24, 48, and 72 h, medium was replaced with fresh medium containing 1/10 volume of cell proliferation reagent WST-1 (Roche Diagnostics) and incubated for 2 h at 37°C before the absorbance at 450 nm was quantified in a spectrophotometer.

Statistics
Statistical analysis was done using the two-tailed Student’s t-test with p = 0.01 considered the upper limit of statistical significance. Data are presented as mean ± SD.

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Author Contributions
Conceived and designed the experiments: JF PK DD. Performed the experiments: JF HS. Analyzed the data: JF PK HS. Contributed reagents/materials/analysis tools: JF PK DD. Wrote the paper: JF.

References
1. Uribe-Botero G, Russell WO, Sutow WW, Martin RG (1977) Primary osteosarcoma of bone. Clinicopathologic investigation of 243 cases, with necropsy studies in 54. Am J Clin Pathol 67: 427–435.
2. Meyers PA, Heller G, Healy J, Hiehs A, Lane J, et al. (1992) Chemotherapy for nonmetastatic osteogenic sarcoma: the Memorial Sloan-Kettering experience. J Clin Oncol 10: 5–15.
3. Provisor AJ, Ettinger LJ, Nachman LD, Kralik MD, Malkey JT, et al. (1997) Treatment of nonmetastatic osteosarcoma of the extremity with preoperative and postoperative chemotherapy: a report from the Children’s Cancer Group. J Clin Oncol 15: 76–84.
4. Goorin AM, Shuster JJ, Baker A, Horowitz ME, Meyer WH, et al. (1991) Changing pattern of pulmonary metastases with adjuvant chemotherapy in patients with osteosarcoma: results from the multimstitutional osteosarcoma study. J Clin Oncol 9: 600–605.
5. Kempf-Bielack B, Bielack S, Degen H, Borschitz D, Berdel WE, et al. (2005) Osteosarcoma relapse after combined modality therapy: an analysis of unsellected patients in the Cooperative Osteosarcoma Study Group (COSS). J Clin Oncol 23: 559–560.
6. Fellenberg J, Bernd I, Delling G, Witte D, Zaltsen-Hinguranaghe A (2007) Prognostic significance of drug-regulated genes in high-grade osteosarcoma. Mod Pathol 20: 1083–1094.
7. Knight RD, Mangum J, Lucas DL, Cooney DA, Khan EC, et al. (1987) Inosine monophosphate dehydrogenase and myeloid cell maturation. Blood 69: 634–639.
8. Itoh O, Kuroiwa S, Atsumi S, Umezawa K, Takeuchi T, et al. (1989) Induction of antitumor and antiviral chemotherapy. Farmaco 51: 457–469.
9. Zimmermann A, Gu JF, Szychala J, Mitchell BS (1996) Inosine monophosphate dehydrogenase expression: transcriptional regulation of the type I and type II genes. Adv Enzyme Regul 36: 75–84.
10. Nagai M, Natsumeda Y, Komoto Y, Hoffman R, Irimo S, et al. (1991) Selective induction of type II inosine monophosphate dehydrogenase messenger RNA expression in human leukemias. Cancer Res 51: 3511–3516.
11. Zurnermann A, Gu JF, Szychala J, Mitchell BS (1996) Inosine monophosphate dehydrogenase expression: transcriptional regulation of the type I and type II genes. Adv Enzyme Regul 36: 75–84.
12. Nagai M, Natsumeda Y, Komoto Y, Hoffman R, Irimo S, et al. (1991) Selective induction of type II inosine monophosphate dehydrogenase messenger RNA expression in human leukemias. Cancer Res 51: 3511–3516.
13. Fellenberg J, Bernd I, Delling G, Witte D, Zaltsen-Hinguranaghe A (2007) Prognostic significance of drug-regulated genes in high-grade osteosarcoma. Mod Pathol 20: 1083–1094.
14. Ratcliffe AJ (2006) Inosine 5'-monophosphate dehydrogenase inhibitors for the treatment of autoimmune diseases. Curr Opin Drug Discov Devel 9: 595–605.
15. Chen L, Pankiewicz KW (2007) Recent development of IMP dehydrogenase inhibitors for the treatment of cancer. Curr Opin Drug Discov Devel 10: 403–412.
16. Sidi Y, Panet C, Wasserman L, Cyjon A, Novogyrodsky A, et al. (1988) Growth inhibition and induction of phenotypic alterations in MCF-7 breast cancer cells by an IMP dehydrogenase inhibitor. Br J Cancer 58: 61–63.
17. Olatu E, Natsumeda Y, Ikogami T, Kote Z, Horanyi M, et al. (1988) Induction of erythroid differentiation and modulation of gene expression by tiazofurin in K-562 leukemia cells. Proc Natl Acad Sci USA 85: 6533–6537.
18. Jayaram HN, Cooney DA, Grusch M, Krupitza G (1999) Consequences of IMP dehydrogenase inhibition, and its relationship to cancer and apoptosis. Curr Med Chem 6: 561–574.
19. Wright DG, Bossalini MS, Waraska K, Osny LJ, Weintraub LR, et al. (1996) Tiazofurin effects on IMP-dehydrogenase activity and expression in the leukemia cells of patients with CML blast crisis. Anticancer Res 16: 3349–3351.
20. Penelas S, Neve V, Ciudad CJ (2005) Modulation of IMPDH2, survivin, topoisomerase I and vimentin increases sensitivity to methotrexate in HT29 human colon cancer cells. FEBS J 272: 696–710.
21. Fellenberg J, Dechant MJ, Ewerbeck V, Mau H (2003) Identification of drug-regulated genes in osteosarcoma cells. Int J Cancer 105: 636–645.
22. Tricot GJ, Jayaram HN, Lapis E, Natsumeda Y, Nichols CR, et al. (1989) Biochemically directed therapy of leukemia with tiazofurin, a selective blocker of inosine 5’-phosphate dehydrogenase activity. Cancer Res 49: 3696–3701.
23. Linke SP, Clarkin KC, Di Leonardo A, Tsou A, Wahl GM (1996) A reversible, p53-dependent G0/G1 cell cycle arrest induced by ribonucleotide depletion in the absence of detectable DNA damage. Genes Dev 10: 934–947.